AFIT/GEE/ENV/95D-01

# EVALUATION OF THE NATURAL BIODEGRADATION OF JET FUEL JP-8 IN VARIOUS SOILS USING RESPIROMETRY

THESIS

James A. Baker III, GS-13

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James A. Baker III, GS-13

Presented to the Faculty of the Graduate School of Engineering

of the Air Force Institute of Technology

in Partial Fulfillment of the

Requirements for the Degree of

Master of Science in Engineering and Environmental Mangement

Member

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Chairman

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Department of the Air Force Civilian, GS-13

December 1995

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Jim Baker

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## Abstract

This research effort used an automated respirometer to evaluate the intrinsic aerobic biodegradation potential of jet fuel JP-8 in various types of natural soils. Four replications of a complete factorial design experiment were accomplished using three levels of fuel and three types of soil in a three by three matrix of treatments. Laboratory microcosms were prepared containing the treatments, using the soils in a close to natural state, and allowed to react for fourteen days. A two-way ANOVA test on the experimental data demonstated a strong positive correlation between the amount of fuel biodegraded with the initial level of fuel and also with the clay content of the soil. Interaction effects were also observed between the two factors. The continuous oxygen uptake rate curves were used to follow biodegradation of the fuel through the various steps of biological growth. The biokinetics of the observed reactions could be inferred from the oxygen rate curves. Analyses of soil nutrient consumption and the predicted ratio of oxygen uptake to carbon dioxide production were also done. Regression analysis demonstrated a significant reduction in nirates in microcosms with higher initial levels of fuel.

EVALUATION OF THE NATURAL BIODEGRADATION OF JET FUEL JP-8

IN VARIOUS SOILS USING RESPIROMETRY

# I. Introduction

## 1.1 Overview

Soil contamination from spilled jet fuel is a common environmental problem at US Air Force installations world wide. Some factors determining the severity of individual problems are the amount of fuel spilled, the soil conditions, and the length of time the fuel has been in the soil. There is a large amount of information in the literature confirming that biodegradation can over time, break down jet fuel in soil into more environmentally safe compounds. The US Air Force has recently switched to a new type of jet fuel, called JP-8. It's biodegradability in soil is not very well known (Dean-Ross, 1992: 219). This research effort used an automatic respirometer to evaluate the intrinsic aerobic biodegradation potential of this new jet fuel in various types of natural soils.

In 1993, the US Air Force began converting its primary turbine engine fuel from JP-4, in use since 1951, to JP-8. The main reason for the conversion was the increased safety factor provided by the lower volatility of the new fuel. Evidence gained from data obtained during the Southeast Asian Conflict showed Air Force aircraft, using the highly volatile JP-4, had higher combat losses than Navy aircraft, which were using a less volatile jet fuel, JP-5. This safety factor was also evident when the frequency and

severity of fuel handling accidents on the ground were compared with fuel type (HQ USAF/LGSSF, 1991). JP-8 is very similar to commercial jet fuel, except for the addition of military additive packages, consisting of various organic and inorganic chemicals functioning as antioxidants, metal deactivators, static dissipaters, corrosion inhibitors, and fuel system icing inhibitors. An additional benefit of lower volatility is the reduction of evaporative hydrocarbon emissions, making storage and handling activities inherently less polluting (and emissions control equipment less costly) with the new fuel.

The US Air Force typically uses over 4 billion gallons of jet fuel annually (Lavin, 1995). Although procedures and equipment are continuously improving, handling that much fuel means leaks and spills are inevitable. Based on data currently maintained in the historical database at their headquarters, the US Air Force had 169 reportable jet fuel spills, amounting to over 114,000 gallons, from calendar years 1992 to 1994 (HQ USAF/CEV, 1995). Figure 1.1 shows the size distribution of these spills. (The actual data show the gradual replacement of JP-4 by JP-8 in the spilled material.)

The potential for environmental contamination from this spilled fuel is significant, even though it amounts to less than one one-thousandth's of a percent of the total amount of fuel handled. In FY95, the US Air Force will spend about $160M on fuel-related remediation projects. These cleanups account for almost 40% of the Air Force's environmental restoration budget, and over half of the total number of the contaminated sites (Furlong, 1995). Often groundwater is also involved, as fuel adsorbed onto soil

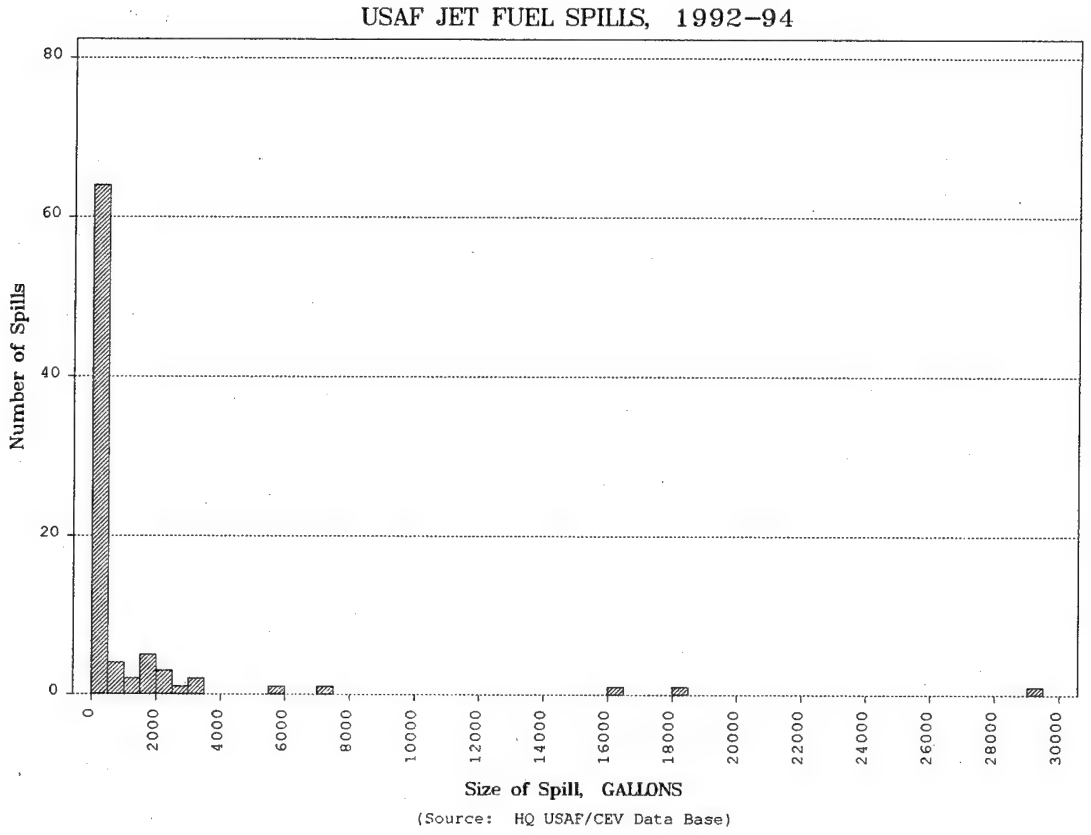



FIGURE 1.1: Distribution of USAF Jet Fuel Spills by Size, 1992-94

particles is mobilized by variations in water table levels. Environmental problems from jet fuel contaminated soils appear to be a byproduct of Air Force operations at installations world wide.

Aerobic biodegradation is an important remediation pathway for fuel contaminated soils. It usually occurs immediately following the contact of fuel with the soil and continues as long as conditions remain favorable for biological growth. Natural attenuation--the purposeful use of this biodegradation--is a remediation option that is gaining favor in

cleanup situations where risks are low. As a starting point, remediation project designers often calculate how much fuel has been degraded naturally since the original spill event and also how much more fuel would degrade over time if undisturbed. This helps them scope the cleanup effort and evaluate biodegradation--natural or augmented--as a candidate method. Biodegradation is an "environmentally friendly" choice because it destroys organic contaminants without generating problem waste products (Rizer-Roberts, 1992: 20).

There are many methods for evaluating aerobic biodegradation in soil. Most involve complex chemical and biological analyses and these often require extensive sample preparation and expensive laboratory equipment. While not necessarily a field method, respirometry, using a continuously-recording respirometer instrument, offers a relatively simple means to evaluate the process of biodegradation using actual contaminated soil samples with a minimum of sample preparation. The continuous nature of the data provided by this instrument offers a view into the biological process that is normally only available by performing frequent sampling and analyses of the soil over the observation period. If interim sampling should be deemed necessary, its value can be optimized by using the trends and events indicated by the respirometer's output to indicate the best time to sample.

## 1.2 Research Objective

The purpose of this research was to evaluate the degradation potential of JP-8 jet fuel in typical soils under natural attenuation conditions using standard respirometry techniques. The effects of fuel concentration and the clay content of the soil on the biodegradation of the fuel were of primary interest. Other analyses and comparisons were made, including evaluation of general biodegradation factors, reconstruction of the biodegradation kinetics, examination of respiration ratios, evaluation of nutrient consumption, and direct measurement of fuel lost to both evaporation and biodegradation. Another goal of the research was to communicate the procedures and results clearly so that others may understand the process, use it themselves, and perhaps further advance the science and practice of environmental cleanup.

## 1.3 Scope

This study simulated initial spill conditions by challenging uncontaminated soils with fresh jet fuel. Soils that have been contaminated for a period of time were not considered. Three different soils were chosen for their variety of physical structure, specifically particle size distribution. The chemical makeup of the soils was not a discriminating factor; however, they were all taken from areas believed to be free of pollution. The soils were kept to as close to a natural state as possible by keeping any processing to a minimum. Fresh jet fuel was introduced to the soil in three concentrations (including none) to assure a minimum amount of biological activity would occur. Aerobic conditions were initially established in the sealed microcosms and

then automatically maintained by the respirometer equipment. Although no attempt was made to reproduce actual spill conditions, the resulting contamination levels could typically be found at a spill site. The equipment configuration allowed two replications of the matrix of treatments (soil types versus levels of fuel) during each experimental run. Two runs were made with the intention of making total of four replications. Experiments were stopped when the biological activity had peaked and then generally stabilized. For both runs, this period was fourteen days. Samples of soil were taken from each microcosm at the beginning and end of each run. Organic vapors coming from each microcosm were trapped for quantification purposes only. The soil macronutrients nitrate and phosphate were measured in each soil sample. No attempt was made to identify the type of biological constituents (bacteria, fungi, etc.) in the soil.

### 1.4 Terms Used in this Study

Biodegradation - The breakdown of organic compounds in nature by the action of microorganisms, such as bacteria, actinomycetes and fungi (Rizer-Roberts, 1992: 18).

Bioremediation - The use of biological processes, either naturally-occurring or enhanced by man-made activities, for the cleanup of pollution, usually in soil or groundwater systems.

JP-8 - A kerosene-based hydrocarbon fuel currently used by the US Air Force as its primary turbine engine fuel.

Macronutrients - The most essential inorganic chemicals required for bacteriological growth in relatively large amounts; the ones considered in this study are nitrogen and phosphorous. Nitrogen is used for building amino acids, nucleic acids, amino sugars, and their polymers, used in cellular structures. Phosphorous is a constituent of nucleic acids, sugar phosphates, and phosphate esters, used in cellular energy transfer (Atlas and Bartha, 1992: 314).

Micro-Oxymax™ - A fully-automated, indirect, closed-circuit respirometer with integrated instrumentation used for recording extremely low levels of oxygen consumption and carbon dioxide production for a wide variety of studies involving bacteria, insects, plants, cell cultures, food, and chemical oxidation (Micro-Oxymax, 1994: 1).

Natural attenuation - The purposeful use of unaugmented biodegradation for cleaning up pollution in soil and groundwater; sometimes referred to as intrinsic biodegradation.

Respirometer - A device for measuring the oxygen uptake and carbon dioxide evolution associated with the activity of biological or chemical systems.

Supercritical Fluid Extraction (SFE) - A process that takes advantage of the principle that certain fluids, in a supercritical state of matter, significantly increase their affinity for organic chemicals. In this research, extremely pure, supercritical carbon dioxide was used to extract organic chemicals (from jet fuel) from contaminated soils.

Thermogravimetric Analysis (TGA) - A procedure for accurately measuring minute losses in weight of a sample of matter as the temperature of that sample is incrementally raised in an inert atmosphere to inhibit combustion.

# II. Literature Review

## 2.1 Background

Intrinsic aerobic biodegradation is an important treatment option for the remediation of hydrocarbon-contaminated soils. The nature and degree of the biodegradation process must be both fully understood and quantified in order to declare it part (or all) of an approved contaminated site restoration scheme. The US Air Force needs information about the intrinsic biodegradation potential of their new jet fuel, JP-8, that it is presently converting to in the United States. The technique of respirometry is one way of providing a wide range of information about the aerobic biodegradation process, especially in soils contaminated with jet fuel.

## 2.2 Jet Fuel Development and JP-8

Ever since the first turbojet engine found its way into a military aircraft, the Air Force has had a need for high-performance turbine engine fuels. America's early experiences with jet fuels was during and just after World War II. The first US jet fuel specifications were influenced by the British, who had developed the first successful turbojet engine on the Allied side. Because gasoline was in short supply during the war, they had used illuminating kerosene. (The first successful jet engine was developed in Germany and it used gasoline as a fuel.) Jet Propellant-1 (JP-1), introduced in 1944, was a kerosene-based fuel with a specified freezing point of -60°C. Because of the extremely low freezing point, only three percent by volume of a typical crude oil could be used to make

JP-1, severely restricting its availability. The need to raise this availability led to JP-2, a wide-cut distillate fuel; however, it never became operational due to its unsuitable viscosity and flammability. The specification for JP-3 was issued in 1947, which provided another wide-cut fuel with a high vapor pressure, similar to gasoline. This increased performance; however, problems with vapor lock and boil-off at high altitude became more frequent. Finally the JP-4 specification, issued in 1951, had a low vapor pressure requirement (2-3 $lbf/in^2$) which solved the boil-off problems while retaining the other desirable performance characteristics. (The Navy specified another fuel, JP-5, in 1953. It is a high flash point kerosene used to satisfy shipboard safety requirements and it remains the Navy's primary turbine engine fuel today. Other special-purpose and experimental jet fuels (JP-6, JP-7, and JPTS) have been subsequently developed for use in special aircraft that operate at extremely high-altitudes. These have special formulations and additives that make them very different from the more commonly-used straight-chained, alkane based jet fuels.) Later refinements to the JP-4 specification permitted the use of cracked petroleum products (larger molecules being split into compounds with smaller molecular weights, expanding the availability of jet fuel components in crude oil) and allowed an even wider cut in the distillation process, to include both the naphtha (gasoline) and kerosene fractions.

JP-4 remained the primary jet fuel until the early 1990's, when the need for a safer and more environmentally friendly jet fuel became an important mission objective of the US Air Force. The combat loss and safety statistics referenced in Section 1.1 were the main

drivers of this change, although the environmental benefits, primarily in the area of volatile air emissions, quickly became evident. Reduced evaporative losses and the need for fewer emissions control devices soon produced tangible economic benefits, as well. Modern jet fuels can be generally described as a mixture of alkanes (straight chained, saturated hydrocarbons having the general formula, $C_nH_{2n+2}$) ranging from seven to sixteen carbon atoms ($n = 7\text{-}16$), and containing a package of additives needed to satisfy the special needs of the military. Appendix A provides additional information about these additives and the physical and chemical characteristics of jet fuel JP-8. Figure 2.1 demonstrates the relationship of jet fuel JP-4 to gasoline and the makeup of JP-8, which more closely resembles kerosene. Information for this section came from a technical report of the Wright Aeronautical Laboratories' Aero Propulsion Laboratory, which also provided the samples of the jet fuel used in this research (Martel, 1987).

### 2.3 Biodegradation

Any form of biodegradation, intrinsic or enhanced by man, has the same basic elements. There must be suitable populations of microorganisms, a carbon source (normally, the contaminant), oxygen or other electron acceptor, adequate nutrients, moisture, and temperature, and a medium within which the biological growth can take place. Remove any one of these, or let one factor range above or below certain limits, and the desired activity stops. The desired activity in biodegradation is the consumption of the carbon source within the medium (soil, water, or air) and the subsequent conversion of the contaminant into more environmentally-friendly compounds.

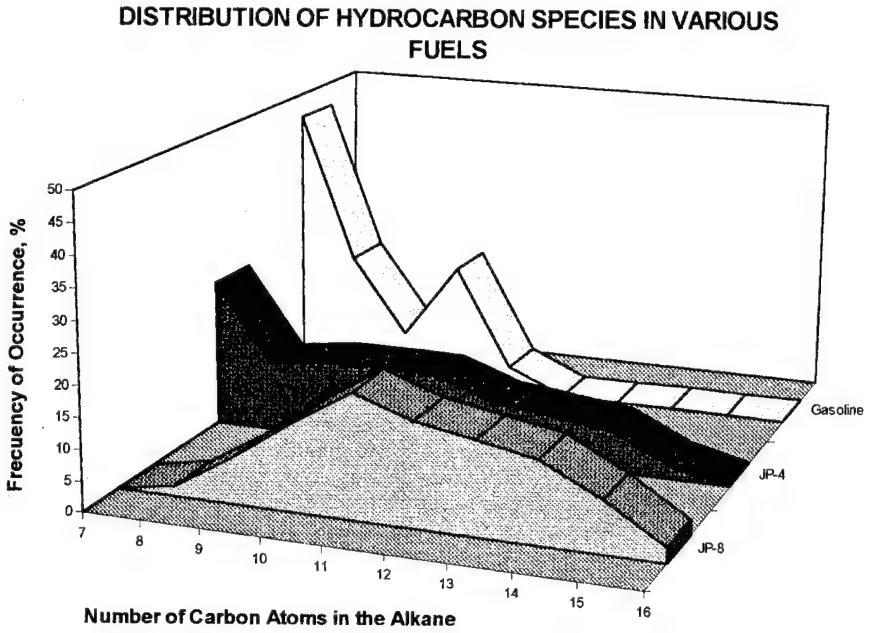



FIGURE 2.1 Distribution of Hydrocarbon Species in Various Fuels

Biodegradation is not the only activity at work on hydrocarbons within a contaminated site. Buschek and Alcantar offer a good description of some of some other common mechanisms that can come into play in addition to intrinsic bioremediation:

> "Advection, dispersion, sorption, and decay [biodegradation] each contribute to the overall attenuation of a dissolved hydrocarbon plume. The effect of advection is to transport dissolved contaminants at the same rate as groundwater velocity. The effect of dispersion is to spread contaminant mass beyond the volume it would occupy due to advection alone, and reduce contaminant concentrations. The effect of sorption is to retard contaminant migration. Two of the conditions for which intrinsic bioremediation is likely to contribute to the configuration of a contaminant plume are a shrinking plume and a stable plume." (Buschek and Alcantar, 1995:109)

The authors go on to say that biodegradation is usually the main cause of a shrinking plume. It can also be the reason that plumes remain stable, as contaminants are removed from soil that is flushed by the periodic vertical movement of contaminated groundwater. The mechanism for aerobic biodegradation of hydrocarbons in soil, the primary focus of this research, centers around the biologically-mediated oxidation of a complex hydrocarbon. A process called complete mineralization transforms the hydrocarbon, in several sequential reactions, into biomass, carbon dioxide and water. Typically 25% of the original carbon goes to biomass synthesis, leaving the remaining 75% of the carbon for the production of carbon dioxide and trace amount of other inorganics (Hinchee and Ong, 1992:1312).

Unless they are under the effect of some other limiting factor, indigenous populations of heterotrophic soil microbes readily degrade most hydrocarbons when they are introduced into the soil matrix.

> "Some of the more common genera of bacteria involved in biodegradation of oil products include Nocardia, Pseudomonas, Acinetobacter, Flavobacterium, Micrococcus, Arthrobacter, and Corynebacterium." (Riser-Roberts, 1992:20)

Biodegradation usually begins following a time lag caused by the microbial populations adjusting to the new food source by producing adequate supplies of new enzymes. Populations that cannot synthesize the enzymes needed to utilize the hydrocarbon source will die off, allowing other more suitable populations to grow. Often these populations will rise and fall in sequential order, as conditions favoring one species enable it to

metabolize the compound part of the way towards complete mineralization. Then other populations use the previous species' metabolites to continue the process. This can also lead to incomplete mineralization, sometimes due to the metabolites themselves causing a toxic effect on the other species.

The kinetics of biodegradation are as varied as the environmental conditions and microbial diversity encountered in nature. Generally speaking, the bacterial growth kinetics are zero order (independent of concentration) when the concentration of substrate (in this case, the hydrocarbon) is high relative to the microbial population. First order kinetics are observed when substrate concentrations are low, and Michaelis-Menten kinetics apply when the substrate concentration is increasing and the rate of biodegradation is changing from being proportional to being independent of the substrate concentration. (Riser-Roberts, 1992:32) Other models, such as with the 3/2 order model, combine several mechanisms into one, in an attempt to capture complexity of the process. (Knaebel and others, 1994:4502)

The alkanes that make up jet fuel are aliphatic hydrocarbons that are readily biodegraded under aerobic conditions by mono- or dioxygenases and cleavage by β-oxidation. The very complex and sequential nature of biodegradation is illustrated by these two processes. Beginning with the terminal methyl group, the alkanes are first transformed by microbial enzymes that use molecular oxygen, into an alcohol and water. This can happen directly, where one atom of oxygen transfers to the alkane and the other is

reduced to water (monooxygenases) or sequentially, where both atoms transfer to the alkane, forming an intermediate compound, that is subsequently reduced to the alcohol and water (dioxygenases). In the next step, the alcohol is further oxidized to an aldehyde and a fatty acid, which forms the starting point for the β-oxidation sequence. Cleavage occurs when this long-chain fatty acid is subsequently shortened by a two-carbon unit through the actions of a series of enzymes. In complete mineralization, the cleavage of two-carbon units continues until the chain is broken completely down and the end products, $CO_2$ and $H_2O$ are all that are left. (Atlas and Bartha, 1992:394)

Skladany and Metting provide some useful generalizations about the biodegradation of aliphatic hydrocarbons:

> "...(1) long-chain n-alkanes are transformed more slowly than short-chain compounds, (2) saturated hydrocarbons are more readily degraded than their unsaturated analogues, (3) the degree of branching is inversely related to the rate of degradation, and (4) recalcitrance is common among highly methylated aliphatic compounds." (Skladany and Metting, 1993:488)

Other authors, Atlas and Bartha, amplify this by saying:

> "n-Alkanes of intermediate length ($C_{10}$-$C_{24}$) are degraded most rapidly. Short chain alkanes are toxic to many microorganisms, but they generally evaporate...rapidly." (Atlas and Bartha, 1992:394)

Jet fuels like JP-8 are a mixture long- and short-chain alkanes ($C_8$-$C_{16}$), contain mostly saturated and unbranched hydrocarbons, and have only contaminant amounts, if any, of the methylated aliphatics. It is not surprising, then, that they biodegrade very readily.

The soil chemistry and structure can significantly affect both the rate and cumulative amount of biodegradation.

> "The ultimate fate of a chemical in soil is the result of several competing processes: biodegradation, irreversible sorption, humification [incorporated into complex organic compounds found in soil], and diffusion into interstitial spaces not accessible to microorganisms." (Knaebel and others, 1994:4507)

Soil particles form a matrix that can capture water, gasses, organic chemicals, and the microbes either between, on the surface, or within, the particles. For these reasons, biodegradation can take place in any of these places, depending on the scale of the spaces and the affinity of the various components for those spaces. A wide range of chemical and physical activities, such as aerobic and anaerobic reactions, can be occurring simultaneously, separated by just a few microns.

Films on the surface of soil particles are generally thought to be the principal sites of microbial activity. (Metting, 1993:19) Because of the colloidal nature of these films, their interaction with soil clay constituents can be quite important. Both positive and negative effects can occur. Clay can concentrate organic and inorganic substrates, enhance the exchange of enzymes with substrates, buffer against wide swings in pH, retain needed moisture, and protect against predators and toxic metabolites. On the negative side, clay can immobilize cells, inactivate enzymes, polymerize certain substrates, and reduce oxygen availability. (Metting, 1993:19)

Many of today's common treatment methods for hydrocarbon contaminated soils, including soil washing, vapor extraction, stabilization and solidification, chemical precipitation, vitrification, thermal desorption, and incineration, simply bind the contaminants in a modified matrix or transfer them from one medium or location to another. Biodegradation is one of the few ways to completely destroy the contaminants. Evidence is mounting that opens this form of treatment to even more complex hydrocarbons, once thought to be recalcitrant to biodegradation. Benzene, toluene, ethylbenzene, xylenes, naphthalene, methyl-napthalenes, dibenzofuran, and fluorene are some compounds that have been successfully treated by aerobic biodegradation. (Rifai and others, 1995:3)

## 2.4 Respirometry

> "Measurement of oxygen consumption is one of the oldest means of assessing biodegradability. .....respirometry is attaining prominence in biodegradation studies and is becoming one of the more suitable experimental methods for measuring the biodegradability and the kinetics of biodegradation of toxic organic compounds by.....soil microbiota...." (Tabak and others, 1989:1)

An important aspect of intrinsic bioremediation is the ability to evaluate the biodegradation that is occurring at the site. The biochemical activities at a site are very complex. For this reason, the more that can be learned about the amount and rate of biodegradation the higher the success in using the process for contaminated site remediation. Respirometry is one method of observing a biodegradation process as it occurs. This creates opportunities for examining the mechanisms at work during critical

changes in the biological process. Dramatic drops (or rises) in biological activity rates can be readily observed and then samples can be taken at these points to evaluate the causes. The total biological activity that occurred over time can also be assessed by the cumulative oxygen uptake.

Respirometry is the study of the activity of biological or chemical systems by measuring their oxygen uptake and carbon dioxide evolution, in other words, respiration. Due to the many interferences and uncertainties with carbon dioxide in experimental studies, the oxygen uptake is a more accurate predictor of biological activity. The carbon in the substrate being studied may not all be converted to carbon dioxide gas--a significant portion is used for the production of biomass. If the gas is produced, it may dissolve into any moisture present, further obscuring the actual amount produced by respiration. Unless severe corrosion or other inorganic oxidation is occurring, the oxygen uptake of a natural system is a good measure of bioactivity.

By continuously monitoring the oxygen uptake of a system, and plotting it over time, the lag phase, exponential growth rate, and total biological growth can be evaluated. If heterotropic biological activity is solely responsible for the oxygen consumption, then the amount of substrate consumed can be calculated by relating the oxygen uptake to theoretical substrate utilization. (Naziruddin and others, 1995:151) Single compounds are often evaluated to obtain reference utilization rate curves. The effects of toxicity can also be measured by introducing a compound with previously-known effects.

Respirometry is often used with sealed batch reactors or microcosms, which makes the technique ideally suited for evaluating volatile organic compounds. By accounting for the microbial growth, substrate use, product formation, and oxygen uptake with known mathematical relationships, the activities within the microcosms can be modeled. Each event can be expressed in terms of at least one of the others and certain coefficients must be quantified (or assumed) in order to obtain a complete model. Some assumptions need to be made about the activities measured by the respirometer if the model is to accurately reflect the biodegradation process: (1) substrate consumption, biomass growth, and product formation are the only events contributing to oxygen uptake, (2) oxygen uptake starts immediately upon initiation of the experiment, that is, no lag time, (3) all the bacteria in the microcosm participate in degradation of the substrate, requiring an enrichment step, where the bacteria are first grown on the test compound as their sole substrate, and (4) the initial concentration of the test compound and biomass should be known accurately. When dealing with volatile substrates, the partitioning between gas and liquid phases must be also accounted for. (Naziruddin and others, 1995:152)

Not all respirometry is done with sealed microcosms. One recent test used an *in situ* test method to measure respiration of ongoing biodegradation in the unsaturated zone of a contaminated soil site. By periodically ventilating the soil with air and then monitoring the depletion of oxygen and the production of oxygen after the air is shut off, respirometer-like data can be obtained. From the oxygen utilization rates, information about the rate of substrate consumption can be obtained. (Hinchee and Ong, 1992:1305)

## 2.5 Summary

Respirometry data has many uses in the bioremediation field. Oxygen uptake information may be used to model hydrocarbon consumption and this can be used to extrapolate the biodegradability of the compound under actual restoration conditions. Care must be taken to assure the conditions used to make these estimates are within the boundaries of the experimentally-derived constants.

# III. Methodology

## 3.1 Overview of the Experiment

This methodology describes how the experiment was set up to gather and analyze the data necessary to meet the research objective. The experiment evaluated the intrinsic aerobic biodegradation of jet fuel (JP-8) in different soils by measuring the respiration of the endemic soil microflora. Various levels of fresh jet fuel were used to assure measurable levels of respiration would occur, and then stabilize, during a fourteen day observation period. Three different soils, selected for their different particle size distributions, were used to represent a wide range of conditions found in nature. Processing of the soils was purposely kept to a minimum, although maintaining *in situ* conditions was not a goal. Samples of the fuel-contaminated soils were placed in clean jars, each one connected to a dedicated monitoring channel of a continuously-recording respirometer. These microcosms were allowed to sit quiescently for fourteen days at ambient room temperatures. The oxygen and carbon dioxide levels in the microcosms' headspaces were measured automatically by the respirometer at regular intervals--typically every eight hours--throughout the period. In each microcosm, the levels of fuel, nitrates, and phosphates were measured in the soil samples before and after each experiment's run. The jet fuel that evaporated in the microcosms during the experiment was also collected and quantified, enabling a mass balance to be done. The respirometer data was used to evaluate the amount and type of biological activity involved in the mineralization of the fuel. The macronutrient consumption is expected to confirm that

biological activity occurred and the direct fuel measurements should also independently confirm the fuel losses due to evaporation and biodegradation. All data were evaluated across the three soil types and relationships with soil characteristics were sought, specifically with regards to particle size distribution.

## 3.2 Statistical Experimental Design

### 3.2.1 General.

Generally accepted sampling methods and laboratory techniques were used to assure the randomness of all material samples and consistency of laboratory analyses. The jet fuel used throughout the test was considered uniform because it was obtained from the single original sample obtained from the Air Force Wright Aeronautical Laboratories and stored in a sealed container. The individual weights of fuel required for each level of treatment were converted to volumes, using the fuel's specific gravity, and dispensed volumetrically, using glass pipettes of known volume and tolerances. The entire specimen of each soil type was gathered at the same time and was thoroughly mixed several times before being divided up and placed into storage containers for later use in the experiments. All in-house laboratory analyses performed for the research were done in duplicate, and often in triplicate, and the results were averaged to account for variation. Contamination in the laboratory was prevented by thoroughly cleaning all equipment, glassware, and implements that came in contact with the samples and rinsing them with distilled water. Careful documentation of each laboratory step was made, in the event that later clarification of results or procedures was needed.

### 3.2.2 Fuel and Clay Affect Oxygen Uptake

The selection of the experimental pattern is the primary tool for assuring the attainment of the objectives of the experiment (Moen and others, 1991: 68). The main purpose of the research is to evaluate the degradation potential of jet fuel in typical soils. Towards this goal, the experiment was designed to evaluate the effects of two main factors, jet fuel concentration in the soil and soil type, expressed as the clay content, on the response variable, cumulative oxygen uptake, which is a measure of the total amount of biodegradation. Two levels of fuel (0.1% and 1% on a dry soil weight basis) were chosen to avoid toxic effects on common soil bacteria and fungi (Riser-Roberts, 1992:33). The third fuel level was zero, chosen to observe background effects of the soils. Of the three soils, Soil C had the highest clay content (29%), followed by Soil A (16%), and Soil B (6%). Each experimental run evaluated the three levels of each factor, in a 3 x 3 factorial design. The resulting nine treatments were arranged in a matrix pattern and their assignments to individual microcosms were randomized. This complete 3 x 3 factorial design enabled every combination of treatments to be tested. Variations in the performance of each respirometer channel were accounted for by randomizing the assignment of treatments to the channel-numbered microcosms. Figure 3.1 demonstrates an example of a typical treatment-to-microcosm assignment pattern for twenty channels (from experiment JIM 1), which yields two replications of the entire treatment matrix including the controls. The amount of oxygen used for biodegradation of the jet fuel in each microcosm was isolated from the background soil respiration by including the

oxygen uptake values from the "zero fuel" microcosms, found in the right-hand columns of the matrix, as a block of treatments.

(1) Nine treatments were developed from the three different levels of the two factors. Each position in the resulting matrix was assigned a number (plus zero, for a control).

(2) A column with two sets of treatments (to fill all 20 channels of the respirometer) was then randomized and matched to a column of respirometer channel numbers.

| SOIL TYPE | JET FUEL CONCENTRATION | | |
|---|---|---|---|
| | 1% | 0.1% | 0% |
| A | 1 | 2 | 3 |
| B | 4 | 5 | 6 |
| C | 7 | 8 | 9 |

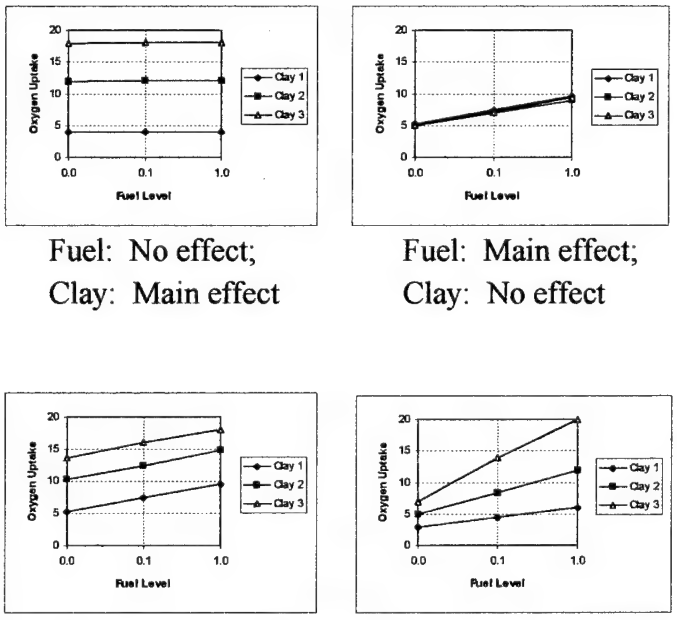
(3) The result was a matrix of treatments that were randomly-assigned to each of the 20 respirometer channels. This enabled two replications of the experiment to be run for each 14-day equipment.

| SOIL TYPE | JET FUEL CONCENTRATION | | |
|---|---|---|---|
| | 1% | 0.1% | 0% |
| A | 9,16 | 3,10 | 5,8 |
| B | 14,18 | 1,11 | 12,15 |
| C | 19,20 | 6,13 | 2,7 |
| Empty microcosms: 4,17 | | | |

FIGURE 3.1 Assignment of Treatments to Microcosms

The particle size distribution of a soil is expected to have an effect on the biodegradation of jet fuel. The level of jet fuel in the soil should also be directly proportional to the total biodegradation observed. The hypothesis to be tested was that there was no interaction between the clay levels and jet fuel levels on the total oxygen uptake. It was expected that there would be an interaction. A two-factor ANOVA test using the F statistic and *p*

*value* was used for these evaluations. Once interaction was proved, a comparison of means was done to determine if any of the treatments were significantly related to one another. It was expected that the means of the zero fuel treatments may be related, however, the means of the remaining treatments were expected to be significantly different, as demonstrated by the Tukey methodology. Figure 3.2 shows the range of possible relationships the two factors could have on the bioremediation.




Fuel: No effect;
Clay: Main effect

Fuel: Main effect;
Clay: No effect

Both have effect;
No interaction

Both have effect;
With interaction

FIGURE 3.2 Range of Possible Effects of Clay and Fuel on Biodegradation

In this part of the experiment, a PC-based statistical analysis package, Statistix® v4.1, performed a two-factor ANOVA routine on the experimental data, partitioning the sums of squares and providing other data used in the test procedure. The total sum of squares

was partitioned into treatment and error components. A level of significance, $\alpha = .05$, was used for this and all subsequent tests, to produce the rejection regions for the null hypothesis ($H_o$). A *p value* less than $\alpha$ would also be grounds for rejection. Statistix® further partitioned the total sum of squares into the interaction sum of squares and the sum of squares of each factor. The interaction sum of squares was used to compute the F statistic [MS(AB) / MSE] used to test $H_o$ that interaction between the two factors does not significantly affect the response variable. If this test led to the rejection of $H_o$, the factors would be interacting to affect the response. A *p value* less than $\alpha$ would also be cause for rejection. The next step in the analysis would be to determine if any of the various pairs of treatments were significantly the same, which provides more insight on the nature of their interaction. A Tukey comparison of means analysis was conducted to determine if each treatment produced an unique outcome or if there were any significant relationships between and among the treatments. Figure 3.3 provides an overview of the above statistical process.

## Tests Conducted in Analysis of Factorial Experiments, Completely Randomized Design, **r** Replicates, **a** x **b** Factors

Assumptions for all tests:

a. The reponse distribution for each treatment is normal.

b. The reponse variance is constant for all treatments.

c. Experiments produce random and independent samples in each treatment.

d. The level of significance for all tests is $\alpha = .05$.

### 1. Test for Factor Interaction

$H_o$: Factors A and B do not interact to affect the response mean.
$H_a$: .Factors A and B do interact to affect the response mean.

*Test statistic:* $F = MS(AB) / MSE$

*Rejection region:* $F \geq F_\alpha$, based on (a - 1)(b - 1) numerator and (n - ab) denominator degrees of freedom {Also reject when *p value* exceeds $\alpha$.}

### 2. Tukey Comparison of Treatment Means

Use MSE from 2-way ANOVA to obtain the variance, s:

$s^2\{D_{hat}\} = 2\ MSE / n$ , where D is the differences between all the means.

Construct a confidence interval using the Student's T distribution, q:

$$D_{hat} \pm T\ s\{D_{hat}\}$$

$$T = \frac{1}{\sqrt{2}} q[1 - a;\ ab,\ (n-1)ab]$$

If the difference between any two pairs of means is greater then one-half the confidence interval, then there is a significant differnce between the means.

(Source: McClave and Benson, 1994: 884-5)

FIGURE 3.3 Procedures for Conducting Complete Factorial Test

### 3.2.3 General Biodegradation Factors

There are many factors involved in the complex biochemical reactions responsible for biodegradation. Some examples are the interactions among the various populations of bacteria and fungi, reactions with the individual components of the fuel, the various metabolic pathways the degradation could take, and metabolic by-products that form and are subsequently consumed. The exact details of these reactions are beyond the scope of this research; however, there is much information that can be inferred from the respirometer's output, especially the oxygen uptake curves. These curves provide a general overview of the biological processes and because the activity in each microcosm can be observed independently, comparisons among the various combinations of soil types and jet fuel levels can be made as the biodegradation progresses.

A characteristic lag time before biological activity begins was expected to be observed for each soil type and level of jet fuel. This would provide insight into the possible toxic effects of the fuel and also the disposition of a particular soil for biodegradation. Similarly, the slope of the oxygen uptake rate curve and the maximum rate should be unique to each soil, providing more information about the kinetics of the biological process. The possible type of kinetics at work (first order, 3/2 order, Monod, etc.) could be inferred, as well as the rate at which the reactions take place and the total amount of activity, which should relate to the total amount of fuel mineralized. Finally, the effects of the various soil types on these factors were of particular interest. All these characteristics were evaluated empirically using known biodegradation research to

provide the best explanations for the observed behaviors. Figure 3.4 illustrates some of the factors that were expected to be observed.

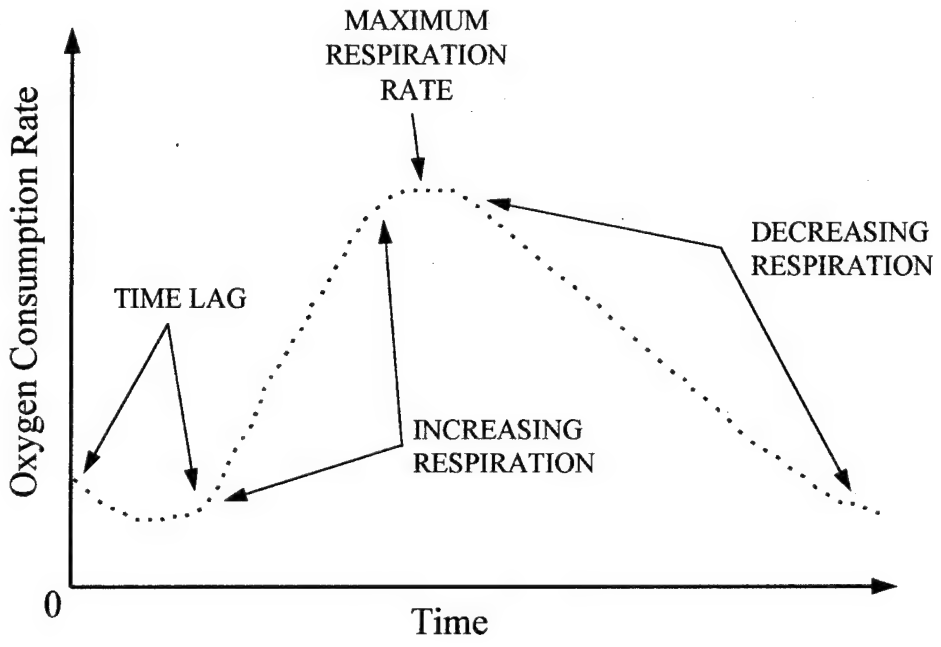
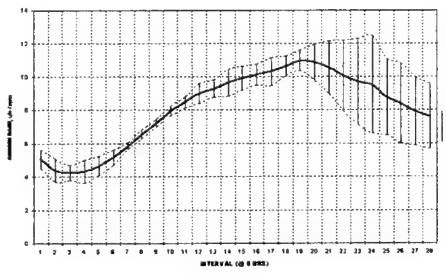



FIGURE 3.4 General Biodegradation Factors

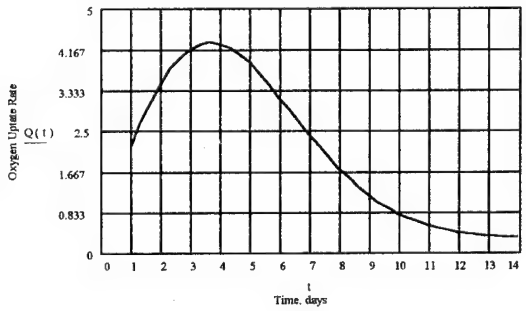
### 3.2.4 Specific Biodegradation Kinetics

It would be useful if the specific type of biodegradation kinetics could be identified from the respirometer's output. To this end, the oxygen uptake rate curves were compared to theoretical models of various type of mineralization kinetics. The curves generated by these models were overlaid with the 95% confidence interval (C.I.) envelope of the sample data means and the fit was empirically evaluated. The 95% C.I. of each of the means of the four replications was constructed using the Statistix® package. The model curves were generated using Mathcad® v5.0+, a PC-based mathmatics package. The

basic equation for each model, expressed in terms of substrate loss versus time, was entered into a Mathcad® template. The first derivative of the equation with respect to time was computed and used with an input vector of time intervals to generate an output vector of substrate loss rates. These were graphed and could be compared directly with the experimental oxygen consumption rates. The parameters of the models were adjusted within reasonable theoretical limits to increase the goodness of fit. A typical equation was the 3/2-order mineralization model: $P = P_0\left(1 - e^{\left(-k_1 t - \frac{k_2 t^2}{2}\right)}\right) + k_0 t$ . In this equation, P is the percentage of the compound mineralized at time $t$, $P_0$ is the percentage of the compound converted to $CO_2$ during first-order metabolism, $k_1$ is a proportionality rate constant ($day^{-1}$), $k_2$ is a linear growth rate term, and $k_0$ is a zero-order rate constant (percent $day^{-1}$). (Knaebel and others, 1994:4502) Figure 3.5 shows an expected sample data curve and also the theoretical model's output.



95% C.I. Sample Data




3/2 Kinetics Model

Figure 3.5 Expected Sample Data and Growth Model Curves

### 3.2.5 Ratio of Oxygen Consumed to Carbon Dioxide Produced

Dodecane ($C_{12}H_{26}$) was selected as the hydrocarbon species most representative of the JP-8 formulation (reference Figure 3.6). A balanced chemical equation for its complete mineralization is: $C_{12}H_{26} + 18.5O_2 \xrightarrow{bacteria} 12CO_2 \uparrow + 13H_2O$ . The number of moles of the hydrocarbon that are oxidized to carbon dioxide by one mole of oxygen were then determined. The ratio of oxygen consumed to carbon dioxide produced is 1.5417 for the complete mineralization. The cumulative amounts of these gasses were precisely measured by the respirometer for each microcosm, producing twenty-four independent estimates for this ratio, using data from each microcosm containing jet fuel. If this mineralization equation accurately predicts the observed biodegradation activity, the mean ratio should equal 1.5417.

Assuming complete mineralization, if 25% of the original carbon went to biomass (Hinchee and Ong, 1992:1312), the $O_2$:$CO_2$ ratio would increase to 2.0556, according to:

$C_{12}H_{26} + 18.5O_2 \xrightarrow{bacteria} 9CO_2 \uparrow + 13H_2O + \text{biomass}$ .

If mineralization was incomplete, the observed ratio would be even higher, due to the carbon that was tied up in intermediate biodegradation products. This process proceeds as a series of conversions, beginning with the formation of a primary alcohol:

$C_{12}H_{26} + O_2 + NADPH_2 \longrightarrow C_{12}H_{25}OH + NADP + H_2O$ .

This is further oxidized into aldehyde and finally, a fatty acid. This is converted to its acetyl coenzyme form: $C_{12}H_{25}OOH$ . The acetyl coenzyme is in turn acted on by a series of enzymes, in a series of steps until an acetyl CoA group is cleaved off and the fatty acid is shortened by a two-carbon unit: $C_9H_{23}-\underset{\underset{C}{\|}}{C}-CoA + CH_3-\overset{\overset{C}{\|}}{C}-CoA$ .

The input of oxygen early into this series of reactions produces the aldehydes and fatty acids but does not result in an out put of carbon dioxide. This would not occur until the acetyl CoA group that was cleaved off is converted to carbon dioxide (and water) through the tricarboxylic acid cycle. Mineralization is complete when all acetyl CoA groups are cleaved off the alkanes and converted to carbon dioxide and water. (Atlas and Bartha, 1992:395)

The distribution of these ratios should approximate a normal distribution because they are sums (from the cumulative data) and they result from many independent replications. A Wilkes-Shapiro/Rankit Plot test was performed to test for normality, then the mean and standard deviation of the observed ratios were calculated. Figure 3.7 illustrates how the resulting plots should appear.

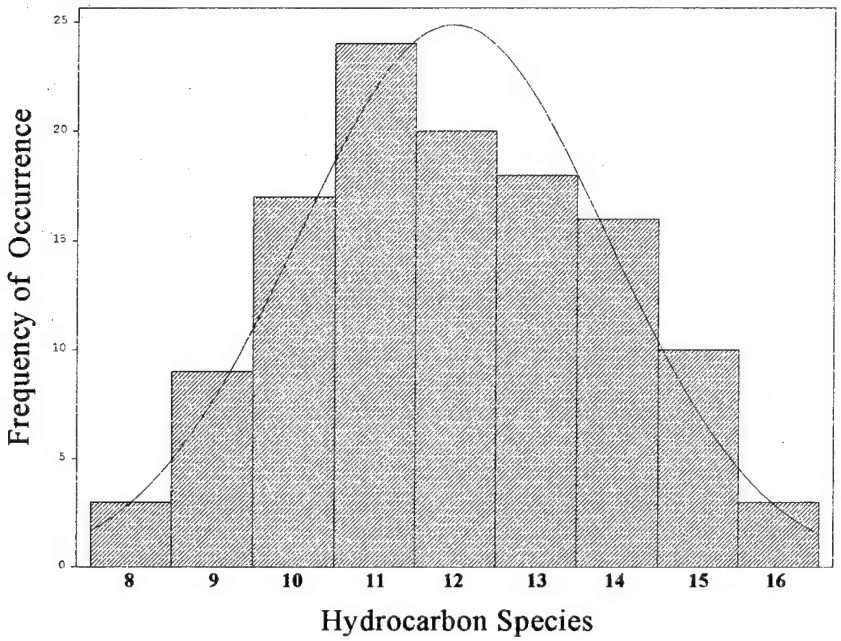
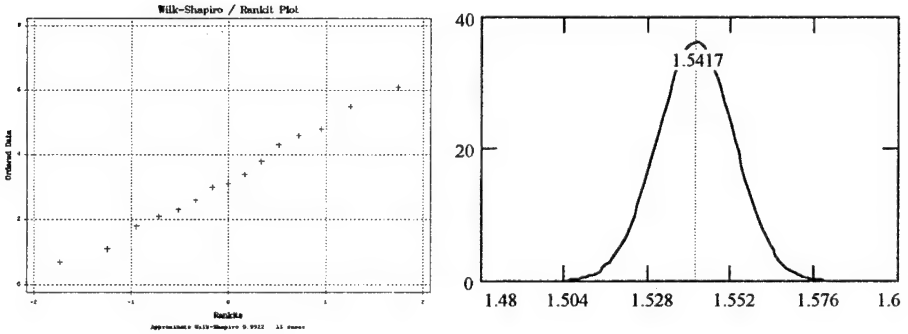



FIGURE 3.6 Typical Distribution of Hydrocarbon Species in Jet Fuel JP-8




Typical Wilkes / Shapiro - Rankit Test Output for Good Normality

Normal Distribution with Small Standard Deviation

FIGURE 3.7 Expected Distribution of $O_2$ to $CO_2$ Ratios

### 3.2.6 Nutrient Levels Affected by Fuel Levels

Biodegradation of jet fuel should reduce the levels of soil macronutrients by an amount proportional to the level of jet fuel added to the soil. The levels of total nitrates and phosphates were measured in each microcosm, as well as their background levels in each soil type. The loss of macronutrients in each microcosm was compared with the corresponding initial levels of jet fuel to determine if there was a statistically significant relationship. In this experiment the factor, jet fuel concentration in soil, was applied in three levels and the response, loss of macronutrients, was observed.

A *linear regression test* was performed to test the hypothesis that increasing levels of jet fuel produce significant, linear increases in the loss of macronutrients. Estimates for the constant ($\beta_0$), slope ($\beta_1$), and error ($\varepsilon$) terms were made from the sample data, using the format, $\mathbf{y} = \beta_0 + \beta_1 \mathbf{x} + \varepsilon$ . In this equation, **y** is the dependent variable representing nutrient loss and **x** is the independent variable representing the level of jet fuel. The estimates were produced by the Statistix® package, as was the *p value* statistic used to test the null hypothesis that the slope of the regression line, $\beta_0$ equals zero, that is, there is no relationship between **y** and **x**. The *p values* provided by the package were compared with the acceptable level of significance, $\alpha = .05$, and values less than this were cause for rejection. Turning to the random error component, $\varepsilon$, it is assumed this has a normal distribution with a mean of zero and a constant variance, $\varepsilon \sim N(\mu, \sigma)$. It is also assumed that $\varepsilon$ and **y** are independent. The package provides an estimation for the

error component, $s^2$, from which the standard deviation, **s**, can be calculated. The package also computed a value for the coefficient of determination, $r^2$, which explained how much of the variation in **y** could be attributed to changes in **x**. Figure 3.8 illustrates the kind of results expected from this test:

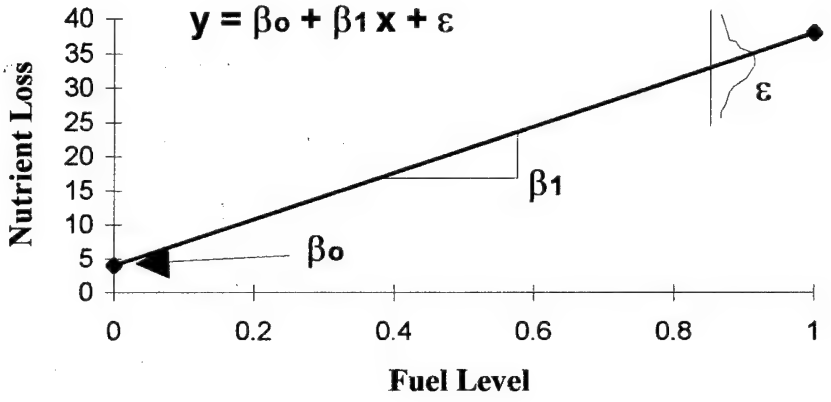



FIGURE 3.8 Expected Relationship Between Fuel Level and Nutrient Loss

### 3.2.7 Predicted Fuel Loss by Respirometer vs. Direct Measurement

The amount of jet fuel biodegraded (and therefore assumed to be completely mineralized) in each microcosm was measured by two independent means: direct measurement and calculated from the oxygen uptake, producing data in sets of pairs, each representing a different treatment. The volume of oxygen uptake was converted into mass of jet fuel consumed by using the general hydrocarbon mineralization equation and molecular weight for dodecane. A mass balance was used to determine the loss of fuel to biodegradation by direct measurement. MASS BALANCE: ORIGINALLY ADDED = LOST (EVAPORATION + BIODEGRADATION) + REMAINED IN SOIL

The fuel lost to evaporation measured by thermogravimetric analysis and the amount remaining in the soil was determined using supercritical fluid extraction (SFE) procedures, respectively. These are described in more detail in later sections.

If the respirometer data accurately predicts the amount of fuel lost to biodegradation, the difference in the calculated mass and directly-measured mass will be zero. The null hypothesis is that there is no significant difference between the means of the experimentally-derived levels and the directly measured levels. (Another way of saying this is the value of the difference between the two means is zero.) If this is true, then the respirometer data accurately predicts the amount of jet fuel mineralized. Figure 3.9 illustrates an example of one possible outcome of this test, where the means of the two sampling populations are slightly different.

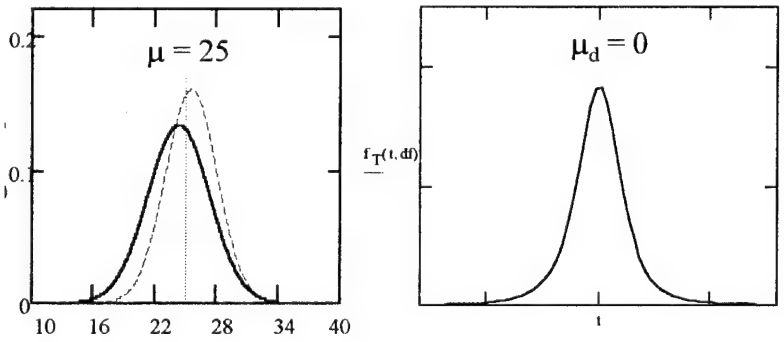



FIGURE 3.9 Setup of a Paired *t* Test for Measured and Predicted Levels

This hypothesis was tested using the paired t test with the *t* test statistic, using the critical value approach. The following are the statistical tools that were used for this test:

The Null Hypothesis, $H_o$: $\mu_{direct} - \mu_{oxygen} = 0$

The Alternative Hypothesis, $H_a$: $\mu_{direct} - \mu_{oxygen} \neq 0$

The Test Statistic, $t_{paired} = \dfrac{\overline{x}_D - 0}{s_D / \sqrt{n_D}}$

where, $\overline{x}_D$ = sample mean of the differences ( $\overline{x}_{direct} - \overline{x}_{oxygen}$ )

$s_D$ = sample standard deviation of the differences

$n_D$ = number of samples in population differences

The Rejection Regions ($\alpha = .05$), $t_{paired} \geq t_{\alpha/2,\, n-1}$ and $t_{paired} \leq -t_{\alpha/2,\, n-1}$

(A comparison of the *p value* produced by the computer package with $\alpha$ also yielded the same result.) Time and equipment limitations allowed up to four pairs of these data to be produced for each of the six treatments with jet fuel, yielding n = 4.

Table 3.1 provides a summary of the statistical analysis design of the data requirements for this research effort.

TABLE 3.1

STATISTICAL DESIGN SUMMARY

| RESEARCH ELEMENT | STATISTICAL APPROACH |
|---|---|
| Level of Jet Fuel in Soil and Clay Content of Soil Significantly Affects Total Oxygen Uptake* | Two-factor ANOVA (w/ interaction)<br>Analysis of Means |
| General Biodegradation Factors: Rate and Other Differences in Dynamics among Various Soils and Fuel Concentrations | Empirical Evaluation of Oxygen Uptake Curves Against Known Biodegradation Performance |
| Specific Biodegradation Kinetics | Fit of 95% C.I. Experimental Data with Theoretical Kinetics Models |
| Ratio of $CO_2$ Evolution to $O_2$ Consumption Indicating General Hydrocarbon Mineralilzation | Wilkes-Shapiro / Rankit Plot Test for Normality<br>Sample Mean & Std. Deviation |
| Level of Jet Fuel in Soil Significantly Affects Loss of Macronutrients in Soil | Regression (Linear or Nonlinear) |
| Jet Fuel Mineralized: Directly Measured and Predicted by Total Oxygen Uptake* are Equal | Paired T Test |

* Total Oxygen Uptake is a Measure of Jet Fuel Biodegradation

### 3.3 Soil Preparation

#### 3.3.1 Purpose

The soils were perhaps the most critical materials used in the experiment because they contain the microflora, nutrients, water, gasses, and structure needed to carry out the biodegradation of the fuel. The three soils used in this experiment were purposely chosen so the experiment would test biodegradation over a wide range of soil characteristics. The most important characteristic for this research was expected to be particle size distribution, especially the clay content. Other soil characteristics, macronutrients (nitrate, phosphate), total organic carbon, ammonia, and pH, were chosen for their possible use in other statistical and empirical studies of the experimental data. All three soils were processed identically, to minimize any differences other than those attributable to the soils themselves. Besides the necessary sieving, other processing was kept to a minimum, so the physical and chemical makeup of the soil remained close to the natural state; however, it was not a goal to reproduce *in situ* conditions.

#### 3.3.2 Soil Collection

The soils were collected at locations that produced three unique samples. The first location, "Soil A", was a wooded area on Wright-Patterson AFB, OH. This area appeared to be completely undisturbed, although it was adjacent to a built up area of the air base. The soil was dark, moist, rich, and contained some root structures. Soil A was sampled on a clear, dry day in early February, with an ambient temperature of about 1°C.

Soil B was collected from a recently-exposed river bottom behind the Huffman Dam, near the air base. Several weeks of rainy weather preceding the sampling produced mild flood conditions behind this flood-control structure. The soil sample was collected from an old drainage channel, which had been flowing full for many days. After the channel dried up, a wet, sandy soil with some fine root structures remained. Soil B was collected on a clear, dry day in mid-May, with an ambient temperature of about 22°C.

Soil C was collected from a residential garden plot in Bath Township, Greene County, OH. Interviews with the owners revealed that although the soil contained a lot of clay, it was quite suitable for gardening because they had been enriching the soil with yard and household compost for many years The soil was very dry and coarse in texture, due to the caking of the clay, and contained some chopped organic material. Soil C was collected three days after Soil B under similar ambient conditions.

The procedures for collecting, processing, and storing the three soils were identical. The collection area was first cleaned of surface debris, then the top 15 cm of soil was removed from a one meter square area and discarded. Soil samples were removed with a clean steel shovel down to a depth of 50 cm and placed in clean, twenty-liter plastic buckets. Processing consisted of forcing the soil through a plastic sieve using a plastic spatula. The sieve, a home swimming pool filter, was 25 cm in diameter and 30 cm long. A grid of 6 mm square openings covered the side and bottom of the cylinder. Once all the soil was sieved to remove stones, twigs, roots, and other foreign matter, it was placed

in a large plastic tub (1 m by 1.5 m x 0.3 m), where it was thoroughly mixed. The soil was then placed in one-gallon (3.785 l), plastic Ziploc™ freezer bags (68 μm thick) and stored in a household-type refrigerator (<4°C) until needed for the experiments.

### 3.3.3 Soil Characterization

The soils' physical characteristics were an important piece of information needed for evaluating the biodegradability of jet fuel in the various soils. A commercial civil engineering laboratory was hired to perform standard soil particle size analyses on the three soils, according to ASTM Method D-422. The results of this analysis are summarized in Table 3.2. The complete laboratory report may be found in Appendix B.

TABLE 3.2

PHYSICAL ANALYSIS OF SOILS

| SOIL | PARTICLE SIZE ANALYSIS* (%) | | | | | | GROUP NAME | GROUP SYMBOL | MOISTURE CONTENT (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Gravel | Coarse Sand | Medium Sand | Fine Sand | Silt | Clay | | | |
| **A** | 3 | 5 | 13 | 24 | 39 | 16 | **Sandy Silt** | ML | 25.3 |
| **B** | 0 | 0 | 40 | 45 | 9 | 6 | **Silty Sand** | SM | 18.3 |
| **C** | 0 | 2 | 9 | 19 | 41 | 29 | **Lean Clay w/Sand** | CL | 18.3 |

*** Method: ASTM D-422**

Source: CTL Engineering, Inc. Report, Project No. 95050791, July 13, 1995

The results of the physical analysis confirmed that the three soils were different enough, especially in their clay content, to demonstrate potential variations in biodegradation.

### 3.3.4 Soil Chemistry

The chemical make up of the soil was also important to the evaluation of jet fuel biodegradation. Only total organic carbon, nitrates, phosphates, and ammonia were analyzed. A complete analysis was not performed, because of the limited scope of this research. Table 3.3 summarizes the soil chemistry and the reader is again directed to Appendix B for the complete lab report.

TABLE 3.3

SUMMARY OF SOIL CHEMISTRY

| SOIL | pH (S.U.) | ORGANIC CARBON (%) | NITRATES (mg/kg, as N) | AMMONIA (mg/kg, as N) | PHOSPHATES (mg/kg) |
|---|---|---|---|---|---|
| A | 7.92 | 7.04 | 280 | 20.3 | 2.80 |
| B | 8.08 | 2.05 | 336 | 11.7 | 2.60 |
| C | 7.82 | 5.01 | 391 | 64.6 | 1.58 |

Source: CTL Engineering, Inc. Report, Project No. 95050791, July 13, 1995

Contrasted with the three soils' physical characteristics, their chemical make ups were remarkably similar, except for the relatively low organic carbon level in Soil B and the relatively high ammonia level in Soil C.

### 3.3.5 Soil Moisture

Soil moisture has a significant affect on the biodegradation of hydrocarbons. An accepted measure of soil moisture, as it relates to biodegradation, is the field capacity of the soil. Field capacity is defined as the percentage of water that can be held in a soil matrix by capillary forces when adequate drainage is provided. (Lyon and others, 1952:190) It is generally accepted that the optimum range of soil moisture for hydrocarbon biodegradation is 40% - 75% field capacity.

The field capacity of the three soils was determined by a laboratory experiment. Each soil was placed in a 15 cm long by 2 cm inside diameter Lucite™ plastic cylinder. The cylinder was tared, and filled with a known weight of 110°C oven-dried soil. A disk of clean filter paper was affixed to the bottom of the cylinder and the soil was lightly packed. The cylinder was suspended in a beaker of distilled water so that the bottom remained at least 3 cm under the surface of the water after the initial drawing up of the water. This apparatus was left like this for twelve hours and then the bottom of the cylinder was pulled out of the water and allowed to drain by gravity for another two hours. The filter paper was carefully removed from the bottom and the cylinder was weighed. The weight of dry soil was subtracted from the resulting weight of wet soil and the amount of moisture at maximum (100%) field capacity was determined.

Using the moisture content of the natural soil as a basis, the moisture content of each type of soil used in the experiments was adjusted to 60% of its field capacity. This level was chosen because it was within the optimum range for biodegradation, but it was also beneficial for the experiments' setup because all three soils were slightly drier--requiring the addition of measured amounts of distilled water to bring them up to 60% field capacity. Adding a precise quantity of water to a sample could be done with more precision than drying the soil to a specific moisture level. By adjusting all three soils to the same field capacity, any variation in biodegradation caused by differences in soil moisture content can be minimized.

### 3.3.6 Microcosm Setup

Each microcosm was prepared identically, except for the addition of the different levels of distilled water (for adjustment of field capacity) and jet fuel JP-8 (to obtain the various treatment levels). The microcosms in this experiment were 250ml Pyrex™ bottles, fitted with lids that had Neoprene™ gaskets lined with Teflon™. Each lid had two quick-release fittings that permitted connections with the 1mm inside diameter plastic tubing used to interface with the respirometer apparatus. Each bottle was first tared, using an Ohaus Triple-Beam laboratory balance, which could be read to 0.01gm. Next, a 100gm weight was added to the opposite pan and soil was added to achieve a balance. Distilled

water was added using a pipette, to adjust each soil's moisture content to 60% of its field capacity. Finally, appropriate amount of jet fuel JP-8 was added by pipette to the bottle, according to the desired level given in the experiment's treatment matrix (reference Figure 3.1). (The fuel sample was obtained from US Air Force Wright Fuels Research Laboratory, Wright-Patterson AFB, OH. A summary of the fuel's characteristics is found at Appendix A.) The resulting microcosm was then mixed thoroughly using a stainless steel laboratory spoon and the bottle was temporarily sealed and set aside while the remaining microcosms were prepared. All prepared microcosms were then connected to the respirometer apparatus and the experiment was begun. The time between the preparation of the first microcosm to the beginning of the experiment run was about ninety minutes.

### 3.4 The Respirometer

#### 3.4.1 Purpose

For this research, a device was needed to measure extremely low levels of oxygen and carbon dioxide that resulted from the respiration of endemic soil microflora following the innoculation of soil with jet fuel. It was also desirable to record respiration data as the experiment progressed, without disturbing the sample microcosms, so the biodegradation of the jet fuel could be observed as it occurred. The apparatus chosen to fill these requirements was a Columbus Instruments Micro-Oxymax™ indirect, closed-circuit respirometer (serial number 94274-1), built by Colombus Instruments International

Corporation, Columbus, OH. It is an integrated system that uses an IBM compatible microcomputer to control all operation, calibration, and data collection functions.

### 3.4.2 Theory and Operation

The Micro-Oxymax™ respirometer, hereafter referred to as the respirometer, uses two state-of-the-art gas sensors to measure oxygen and carbon dioxide. By using two "expansion interface" devices, the respirometer can monitor up to twenty microcosms at one time. The respirometer's control system circulates the air in the headspace of each microcosm through the sensors and then returns the air to the microcosm in a closed loop design. By periodically measuring the two gasses of interest in this air, both rate and cumulative production (or consumption) are recorded. The respirometer measures the air in a reference chamber prior to each microcosm measurement to reduce the sensitivity of the data to ambient conditions and to increase the reliability of the data through frequent recalibrations. The sensors can be purged with fresh air between each microcosm reading to reduce interference between channels, or cross-talk. If the change in gas concentration in a microcosm between measurements exceeds a preset value, the microcosms can be refreshed with a preset volume of ambient air, to prevent oxygen depletion or carbon dioxide toxicity from affecting the biological activity. The oxygen sensor operates as an oxygen battery (that is, a fuel cell) measuring oxygen directly. The carbon dioxide sensor is a single-beam nondispersive infrared device. The respirometer operates from a software-controlled algorithm which has a large set of user-selectable parameters and options that configure the system to each experiment's needs. Once the

microcosms are connected and the equipment is started, the respirometer conducts the experiment and records the data automatically.

Immediately following startup, the respirometer measures the volume of the headspace in each microcosm for later use in its data calculations for the two gasses. Next, baseline oxygen and carbon dioxide concentrations are measured in the reference chamber and in each microcosm. Before taking each reading, the air is circulated in the sensor loop for a period of time calculated from the volume of each microcosm, to assure gas concentrations have stabilized within the closed measuring loop. After a preset interval (time between samples), the measurement sequence is repeated. The change in observed concentrations provides both the rate of change in the volume and the incremental change in the cumulative volume of each gas during the interval. Volumes are computed from concentrations by using the ideal gas law and the various parameters measured by the system. These data are recorded automatically for each interval by a data logger. Many major sources of variation are accounted for by alternately passing the air in the reference chamber and the air in the microcosms through the sensors. The air in the reference chamber provides a stable point of reference because it does not undergo changes due to biological activity but it does experience all the other changes in sensor stability, temperature, and barometric pressure that the air from the microcosms does. The temperatures in the reference chamber and the microcosms are continuously monitored and incorporated into the calculation algorithms. Corrections are also made for the consumption of oxygen by the oxygen sensor. If the microcosms are refreshed

with outside air between samples, then the respirometer once again makes baseline measurements for the next interval--otherwise, it uses the previous samples' data for this purpose--and the cycle begins again.

The preceding description of the equipment and its operation was condensed from the respirometer's operating manual (Micro-Oxymax, 1994).

### 3.4.3 Experiment Setup

The physical setup of each experiment's run was identical. The microcosms were connected to their appropriate channel on the respirometer, using the moisture and organic vapor collection tubes described in later sections. They were placed in a large, polyurethane-insulated picnic cooler and covered with a wool blanket to exclude light. Figure 3.10 provides a schematic diagram of the basic experiment setup.

Before the experiment was started, a series of equipment tests were performed to check the respirometer and the external apparatus connected to it. The gas sensors were calibrated by first purging them with nitrogen gas to obtain a zero reading, and then circulating a calibration gas to make the final span adjustments. The calibration gas used was a "primary standard" gas from Liquid Carbonic Company and contained 0.501% carbon dioxide and 20.4% oxygen, as reported on the cylinder's label. The calibration procedure brought the sensors up to the rated system accuracy of ±0.002% over the range

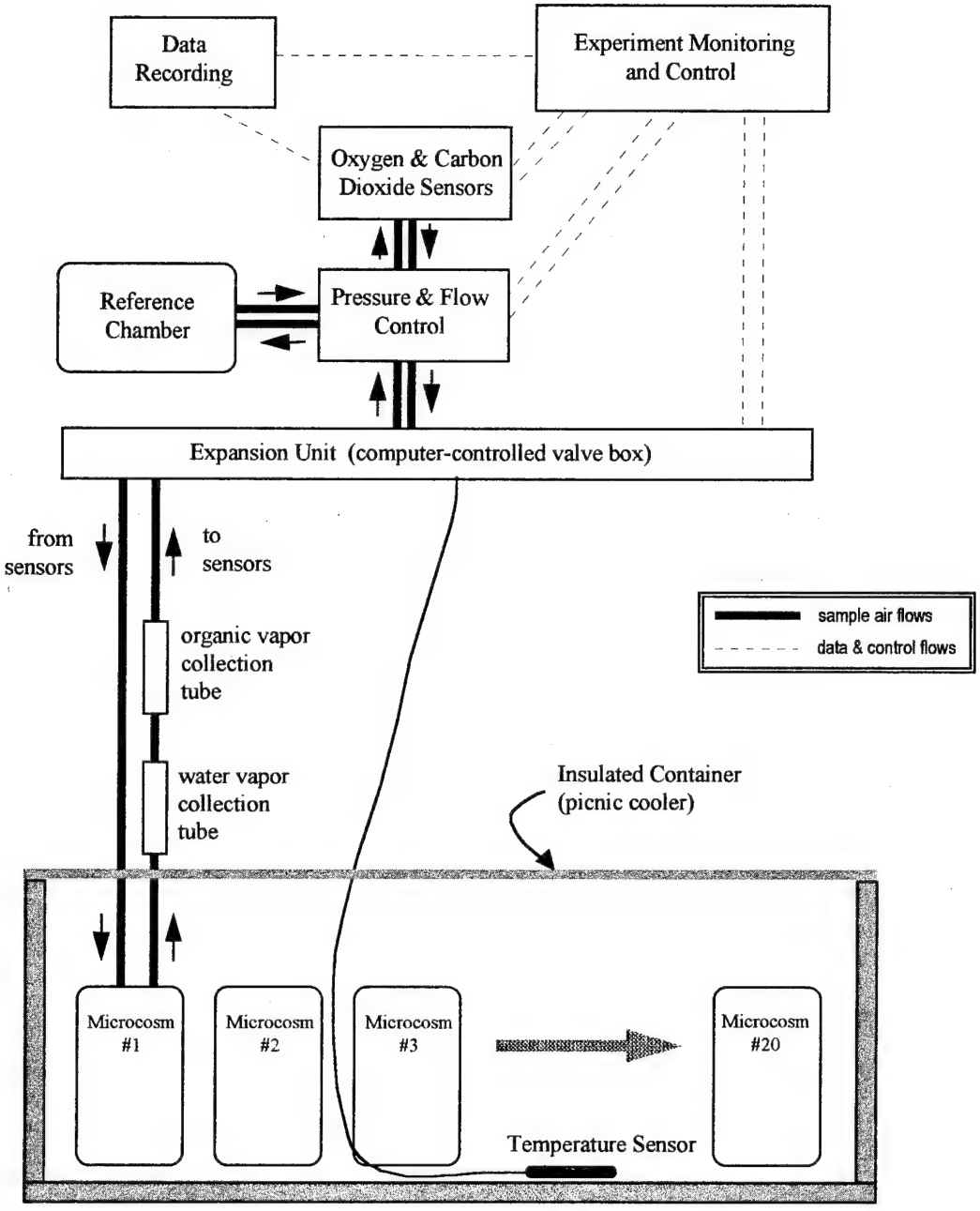



FIGURE 3.10 Schematic Diagram of Experiment Setup

0-0.8% carbon dioxide and ±0.01% over the range 19.3-21.5% oxygen. All valves, orifices, pressure sensors, and other critical devices were checked using the internal test programs of the respirometer. Leak tests were done on all internal and external sampling loops, including the sensors and the microcosms. This automated leak test feature proved invaluable for troubleshooting the many tubing connections in the experiment's setup. After all the tests were successfully completed, the experiment could begin.

#### 3.4.3.1 Trial Runs

Several trial runs were made with the respirometer before conducting the actual research. This was done because it was a brand-new instrument and there was no previous in-house experience with it. These early runs were valuable because they increased the knowledge of both the instrument and the experimental setup. Some of the early problems stemmed from using only microcosms that were totally empty or filled with material having little potential for biological activity. The instrument returned some erratic outputs, especially in the oxygen readings. Due to the extremely fine sensitivity of the oxygen sensor and the auto-scaling feature of the data recorder, these aberrant readings turned out to well below the noise level of the data from actual biological activity. Another early problem stemmed from moisture entering the apparatus. There were two causes for the moisture, one was from an improperly connected refresh-air drier and the other was from the microcosms, which will be described in the following section. A similar problem with activated charcoal powder entering the equipment will also be described below.

Once the setup and the apparatus became more familiar, there were several successful trial runs using actual jet fuel contaminated soil. These runs demonstrated the ability of the respirometer to capture the required data and highlighted the adjustments that were needed in the experimental setup. An especially useful outcome of these tests was the optimum range of jet fuel concentrations that would consistently produce biological activity. An important lesson learned from these tests was that the sensors must be purged between samples. The random assignment of treatments to respirometer channels might place a highly-active microcosm in a channel immediately adjacent to an inactive one (for example, a control), creating a potential problem with cross-talk.

#### 3.4.3.2 Moisture Collection

Moisture control in the experiment was a problem. Liquid water had to be prevented from entering the respirometer system because of the interference and corrosion effects it could have on the valves, tubing, and sensors. Humidity within the sensors also had to be minimized or their accuracy can be adversely affected. The respirometer apparatus was supplied with two air dryers, the first on the outside air inlet, used to refresh the microcosms, and the second in the sensor measuring loop. The air drier on the outside air inlet used a packed column of Drierite©--a proprietary mixture of calcium sulfate and cobalt chloride--that indicates its status with a color change from blue (dry) to red (wet). Drierite© was dried in an oven and reused. Two redundant air dryers in the sensor loop were alternatively switched, allowing the absorbent in the unused one to be changed

while an experiment was running. These dryers were a packed column containing magnesium perchlorate, which was used once and discarded.

The microcosms were also a source of moisture, resulting from evaporation of soil moisture and from the water formed as part of the mineralization of hydrocarbons (ref. equation, Section 3.1.1). Each microcosm was connected to the respirometer apparatus by a supply and return line, which allowed the air in the headspace to be circulated through the sensors. The potential moisture problem was solved by the inclusion of a moisture-absorbing tube in each microcosm's return line. These tubes were Pyrex™ glass, 0.6 cm inside diameter by approximately 8 cm long, filled with magnesium perchlorate with a trace of cobalt chloride crystals to indicate moisture. The absorbent was held in place with disks of filter paper and one-hole, rubber corks at each end. These tubes adequately removed the moisture originating from the microcosms, as evidenced by the lack of condensation in the clear observation tubes, and by the frequent replacement of the tubes required during the experiments' runs.

#### 3.4.3.3 Organic Vapor Collection

To achieve a mass balance of the jet fuel, it was necessary to account for the amount lost to evaporation. This can be a significant portion of the entire amount, according to the available literature (Dean-Ross and others, 1992: 225). To satisfy this requirement, organic vapor collection tubes of the same design as the moisture collection tubes were used. These collection tubes were filled with granulated activated carbon. The initial

particle size distribution of this carbon proved to be too large for the thermogravimetric analysis equipment used in the subsequent analysis, as will be discussed in a later section. The solution was to grind the charcoal using a mortar and pestle, then pass it through a 1 mm opening sieve, made from steel window screen. The charcoal retained on the sieve was used in the vapor tubes. (An early attempt using unshaved, ground charcoal without proper filters in the tubes resulted in charcoal powder being drawn into the apparatus. This required a major cleaning and overhaul of the equipment at the factory. A positive outcome of this was an increase in knowledge of the respirometer apparatus gained from the experimenter's participation in this process.) .As with the moisture tubes, filter disks and corks completed these tubes. The organic vapor collection tubes were placed immediately downstream of the moisture collection tubes in each microcosm's return line. (Neither collection tube type was used with the two empty jars included in each experiment. An in-line moisture trap supplied with the respirometer was used with these "zero reference" microcosms.)

#### 3.4.3.4 Temperature Control

Ambient room temperatures were used during the experiment because equipment was not available to provide precise temperature control of the microcosms. This was not deemed a major problem for the following reasons: 1) the temperature of the microcosms was continuously measured by the respirometer apparatus, 2) the building containing the experiment was of heavy masonry construction with no forced-air air conditioning (experiments were conducted in summer), which would minimize rapid

changes in temperature, 3) the microcosms were placed in a polyurethane foam insulated picnic cooler and covered with a wool blanket, which excluded light and further moderated temperature changes, and 4) temperature variations affected all microcosms equally. The only observed effect was the difference in ambient temperature ranges between the two separate runs of the experiment. This difference may have changed the rates of biodegradation slightly, and this will be discussed in Chapter 4.

## 3.5 Data Collection

### 3.5.1 Electronic Records

The respirometer measured (or calculated) the following data at every interval for each microcosm, using its built-in data logging function: % oxygen, % carbon dioxide, oxygen consumption rate (µl/min), cumulative oxygen consumption (µl), carbon dioxide production rate (µl/min), cumulative carbon dioxide production (µl), temperature (°C) from probes placed within the microcosms' enclosure, and RER (respiratory exchange ratio--the ratio of carbon dioxide production to oxygen consumption). These data were recorded on the hard drive of the controller computer and were later copied onto a floppy diskette for subsequent use. (The respirometer also prints out a report as the experiment progresses; however, these data are only used for diagnostic purposes, if required.)

Much of the experiment setup information was recorded directly onto a microcomputer spreadsheet program. This enabled certain data to be entered, calculations to be performed, and information to be immediately available. The weighing of the charcoal

in the organic vapor tubes was recorded this way, for example. This kind of data appears throughout the various data appendices to this document.

### 3.5.2 Laboratory Procedures

#### 3.5.2.1 Sample Collection and Preservation

The basic soils used for this experiment were stored under refrigeration (< 4°C) in the dark until ready for use. Once the experiment run was over, samples of the soils from each microcosm were collected in duplicate, placed in glass or plastic jars, and frozen (< -8°C). Organic vapor collection tubes were also sealed and frozen (< -8°C). At the end of each experiment run, "time equals zero" soil samples were made up for each of the three soil's two levels of treatment that contained jet fuel. These samples were prepared identically to their respective soils in the microcosms and represented the soils conditions at the beginning of the experiment. These samples were prepared this way so that all soil samples would spend the same time in the freezer and therefore minimized any variations among samples caused by sample storage. It was judged that the variations in the soil sample preparation processes were very small, compared with the possible changes that may occur in the jet fuel inoculated soil after spending an additional fourteen days in the freezer. Once the experiment was complete, all samples would then experience the same preservation conditions.

### 3.5.2.3 Nutrient Analysis

Analyses for the two nutrients of interest, nitrate and phosphate, were done on all soil samples using a commercial laboratory analysis system made by the Hach Company of Loveland, CO. The system used a digitally-controlled spectrophotometer (Model DR/2000, serial number 940800031160) and a series of "single-shot" tests, using premeasured reagents. Although these analyses were claimed by the manufacturer to be consistent and reproducible, at least two replications of each analysis were done to verify the precision of the techniques and improve the accuracy of the results.

Both tests began with a distilled water soil extraction procedure that used a polyelectrolytic flocculating agent to bind the soil particles together, so they would settle out of suspension easily. Since the nitrate ion is soluble in water, no other extraction agents were needed. In addition to the flocculating agents, the phosphate extraction procedure used a proprietary acid/fluoride method.

The nitrate test used a cadmium salt which reduced the nitrate to nitrite, which was then detected by using a sensitive chromotropic acid indicator. The resulting transmittance of 500vm wavelength light was proportional to all the nitrite which was converted from the nitrate in the sample.

The phosphate test used sodium molybdate which formed a complex with the phosphate ion, which was then reduced by ascorbic acid which formed a heteropoly blue species.

The intensity of the blue color was then read by the spectrophotometer using 890vm wavelength light. A summary of the Hach™ procedures and the reagents used in these tests is found in Table 3.4.

TABLE 3.4

SUMMARY OF HACH™ PROCEDURES

| PROCEDURE | REAGENT NAME | HACH CAT. No. |
|---|---|---|
| Nitrate | | |
| Soil Extraction | Nitrate Extraction Powder | 14556-46 |
| NO3 to NO2 Conversion | NitraVer 6 | 14119-66 |
| Chromotropic Indicator | NitraVer 3 | 14065-66 |
| Phosphate | | |
| Soil Extraction | Soil Extractant 1 | 14324-98 |
| Indicator | PhosVer 3 | 2125-68 |

### 3.5.2.3 Organic Vapor Loss Analysis

The organic vapors from the evaporation of jet fuel in the microcosms were collected on activated charcoal as described in Section 3.3.2.2. The carbon was removed from the collection tubes and individual grains were selected randomly and placed in a Perkin-Elmer Thermogravimetric Analyzer (TGA) for evaluation. The TGA raised the temperature of a weighed sample from ambient to up to 900°C at a preset rate and periodically records the sample weight. All this was done in an inert atmosphere of nitrogen gas to prevent combustion of the sample.

The total weight of both water and organic vapor was quantified in the charcoal sample by evaluating a characteristic behavior observed in the TGA output. As the temperature began to rise above ambient, the percent weight loss increased until the temperature passed 100°C, where the rate of loss slowed down dramatically to a characteristic background level. Then, if organic vapors were present, the rate of weight loss began to increase again, starting at around 200°C. The weight loss rate usually slowed again to a background level before the temperature reached the instrument's maximum of 900°C. The percent weight loss of water and organic vapor in the sample, was quantified by measuring the difference in percent weight loss from the beginning to the end of each of the curve's inflection points. The total weight loss can be calculated from the original sample size. Figure 3.11 illustrates the weight loss phenomena and characteristic curve of the TGA analysis of the activated carbon from this experiment. The actual TGA data and curves used to quantify the organic vapors are found in Appendix F.

#### 3.5.2.4 Fuel Loss in the Soil Analysis

To complete the mass balance on the loss of fuel in the microcosms, a direct measurement of the fuel remaining in the microcosm soils was done. The difference between the original weight of fuel inoculated into the microcosms and the sum of the fuel evaporated and the fuel remaining in the soil was equal to the amount of fuel lost to biodegradation. The means for measuring the fuel remaining in the soil was the

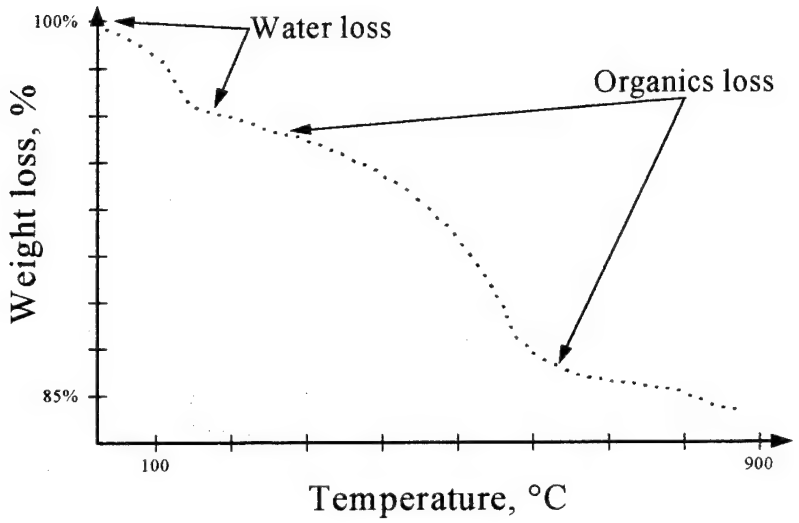



FIGURE 3.11 Characteristic TGA Curve for Activated Charcoal with Water and Fuel

supercritical fluid extraction (SFE) technique. SFE depends on the principle that supercritical liquid carbon dioxide has a great affinity for organic chemicals. In a SFE extractor apparatus, the supercritical fluid was washed through the soil sample, picking up the organics (and the water), and then as it exits, the carbon dioxide evaporates, leaving behind the materials it extracted for collection and quantification.

This procedure was done for selected microcosms by the Chemistry Department at Wright State University.

# 4. Findings and Analysis

## 4.1 Experimental Results

### 4.1.1 Fuel and Clay Levels Affect Oxygen Uptake

Table 4.1 gives the results of the ANOVA test on the experimental data.

TABLE 4.1

RESULTS OF ANOVA TEST ON FACTORS FUEL AND CLAY

| $H_0$: (NULL HYPOTHESIS) | F STATISTIC FOR TEST | F STATISTIC FOR REJECTION | DECISION |
|---|---|---|---|
| Factors Do Not Interact to Affect Responses | **MS(AB)/MSE = 17.234** | **if > $F_{\alpha,4,27}$ = 2.73** | **Reject $H_0$** |

Analysis showed that the factors significantly interacted to affect the response, gross oxygen uptake. Figure 4.1 demonstrates the relationships and interactions graphically.

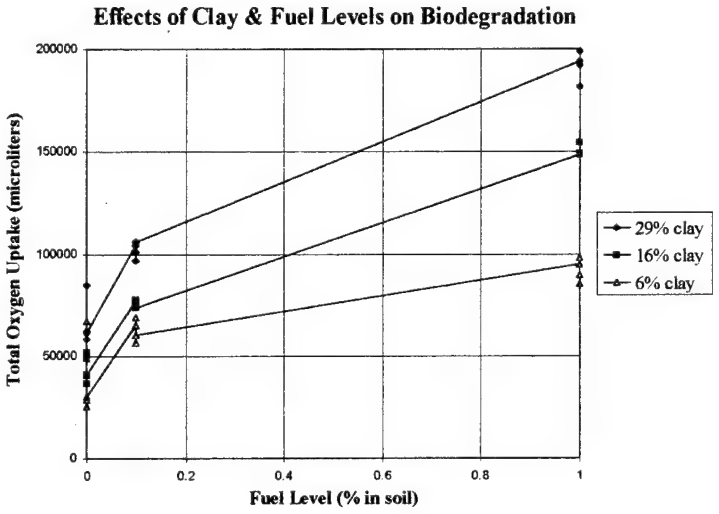



FIGURE 4.1 Effects of Clay and Fuel Levels on Biodegradation

Increasing levels of jet fuel produced increasing levels of oxygen consumption, as did increasing levels of clay. Interaction between the factors was demonstrated as soils with higher levels of clay produced proportionately higher levels of oxygen consumption at the higher levels of jet fuel. This confirmed again that each treatment produced an unique response. Details of the ANOVA process can be found in Appendix D, along with the respirometer data used in the tests.

Table 4.2 shows the results of the Tukey pairwise comparison of the means at the various levels of fuel, further illustrating the interactions, especially at the higher level of fuel. Soil C had the highest clay content (29%), followed by Soil A (16%), and Soil B (6%).

TABLE 4.2

TUKEY PAIRWISE COMPARISON OF MEANS BY THE FACTOR FUEL

| PAIR (@FUEL LEVEL) | SIGNIFICANT DIFFERENCE? |
|---|---|
| CB, 0% | N |
| CA, 0% | Y |
| AB, 0% | N |
| CB, 0.1% | Y |
| CA, 0.1% | Y |
| AB, 0.1% | N |
| CB, 1% | Y |
| CA, 1% | Y |
| AB, 1% | Y |

Oxygen consumption was used to quantify biodegradation by applying the previously described theoretical relationship where 18.5 moles of oxygen are needed to mineralize one mole of dodecane. Table 4.3 tabulates the respiration rates and hydrocarbon degradation rates of the three soils for various levels of jet fuel.

TABLE 4.3

QUANTIFICATION OF BIODEGRADATION

| Soil, % JP8, % Clay | Mean Total Oxygen Cons, ul | Mean Total Oxygen Cons*, mol | Dry soil kg | Respiration Rate** ul/min/kg | Total Fuel Consumed ***,ml | Original Qty Fuel Added, ml | % Lost to Biodeg |
|---|---|---|---|---|---|---|---|
| C, 1, 29 | 185221 | 0.0076 | 0.0862 | 154.4 | 0.0572 | 1.3726 | 4.17 |
| C, 0.1, 29 | 98426 | 0.0040 | 0.0862 | 82.0 | 0.0162 | 0.1373 | 11.80 |
| C, 0, 29 | 64134 | 0.0026 | 0.0862 | **53.4** | | | |
| | | | | | | | |
| A, 1, 16 | 145246 | 0.0059 | 0.0798 | 130.8 | 0.0483 | 1.2667 | 3.82 |
| A, 0.1, 16 | 74015 | 0.0030 | 0.0798 | 66.6 | 0.0147 | 0.1267 | 11.61 |
| A, 0, 16 | 42881 | 0.0017 | 0.0798 | **38.6** | | | |
| | | | | | | | |
| B, 1, 6 | 89355 | 0.0036 | 0.0845 | 76.0 | 0.0249 | 1.2537 | 1.99 |
| B, 0.1, 6 | 60995 | 0.0025 | 0.0845 | 51.9 | 0.0115 | 0.1254 | 9.20 |
| B, 0, 6 | 36581 | 0.0015 | 0.0845 | **31.1** | | | |
| | | | | | | | |
| empty | 924 | | | | | | |
| * - reduced by amount in empty microcosm | | | | | | | |
| ** - shaded figures are the background respiration rates | | | | | | | |
| *** - predicted from O2 consumption, adjusted for background respiration | | | | | | | |

The information in the table is also presented graphically in the figures that follow. Note that the background respiration rates followed the same pattern as the hydrocarbon respiration rates and that background respiration also varies directly with the clay content of the soils. Figure 4.2 shows the respiration rates of each of the three soils for the two levels of jet fuel used in the experiment and the background respiration rates.

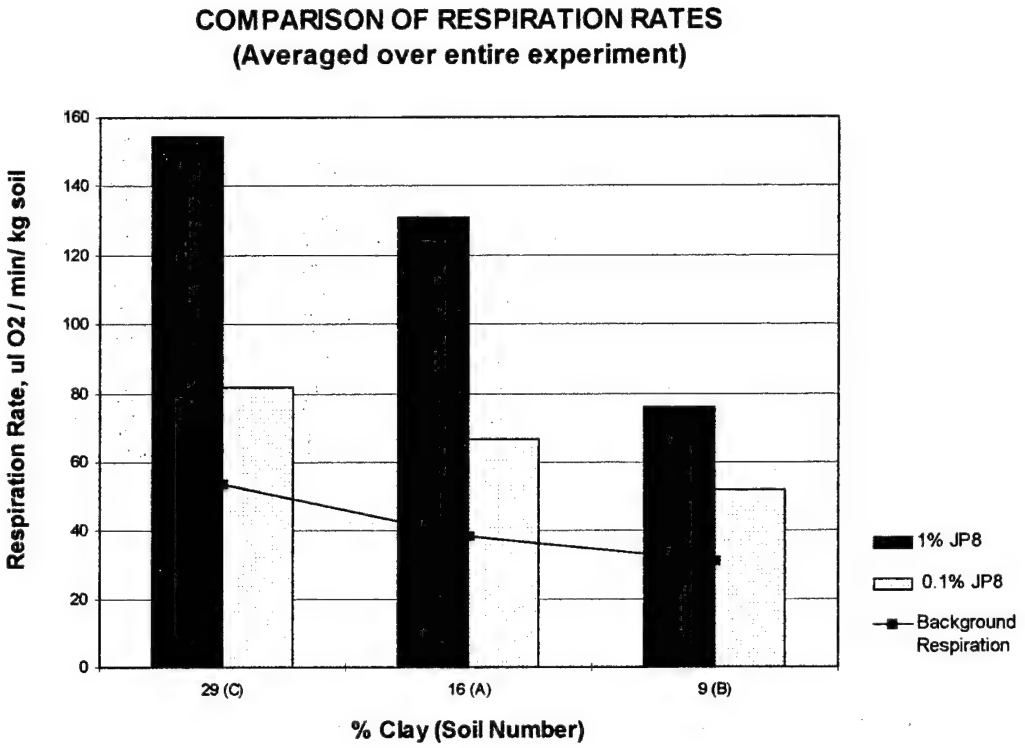



FIGURE 4.2 Respiration Rates

Figure 4.3 illustrates how a high initial level of hydrocarbon in any soil causes a correspondingly high average degradation rate. The comparison of the rates with the soil type shows a similar pattern, however the relationship with the clay content is not as strong, as Soils A and C have almost the same rates for both levels of fuel.

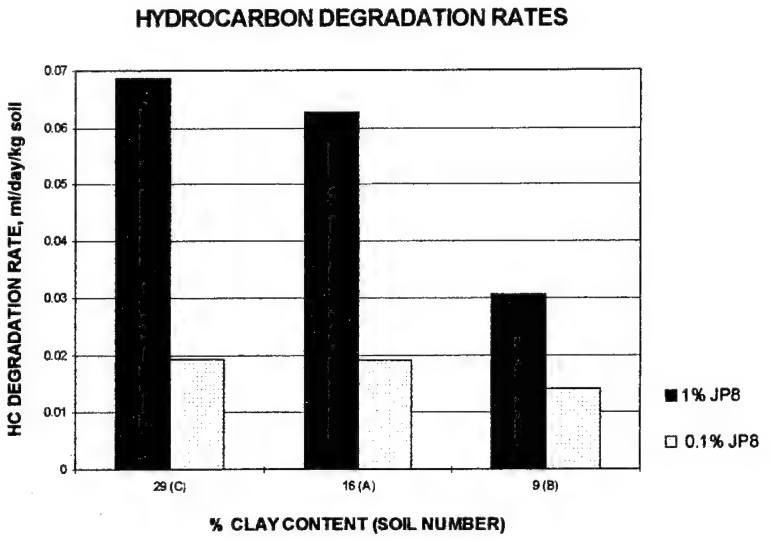



FIGURE 4.3 Hydrocarbon Degradation Rates

Figure 4.4 shows how the relative amount of fuel lost to biodegradation follows the same pattern with respect to the soil type, but, the relationship is reversed with respect to fuel.

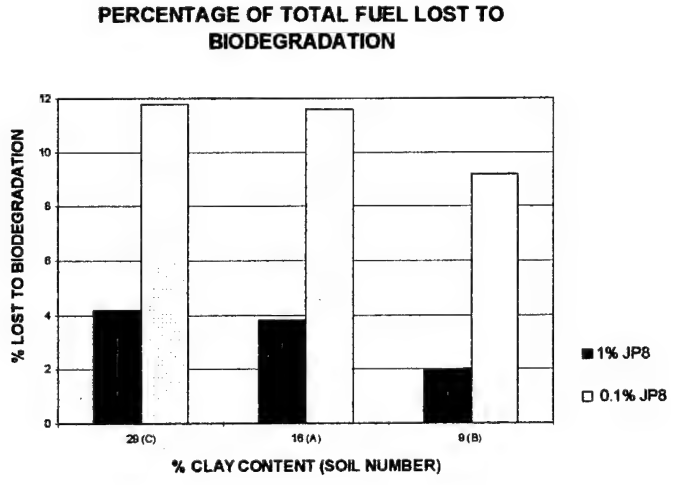



FIGURE 4.4 Fuel Lost as Percentage

### 4.1.2 General Biodegradation Factors

The three soils demonstrated very characteristic behaviors with respect to the main elements of their oxygen respiration curves. The curves all began with an initial lag time, where no conclusive oxygen consumption could be measured. This was followed by increasing respiration rates, leveling off at a maximum, and then ending with a declining rate. Each soil exhibited an unique, repeatable pattern of these characteristics. All the respirometer curves can be found in Appendix E. Figure 4.5 shows the characteristic oxygen respiration rate curves for Soil A, the medium clay forest soil. The next three curves were constructed from the mean respiration rates of all four replications of the experiment.

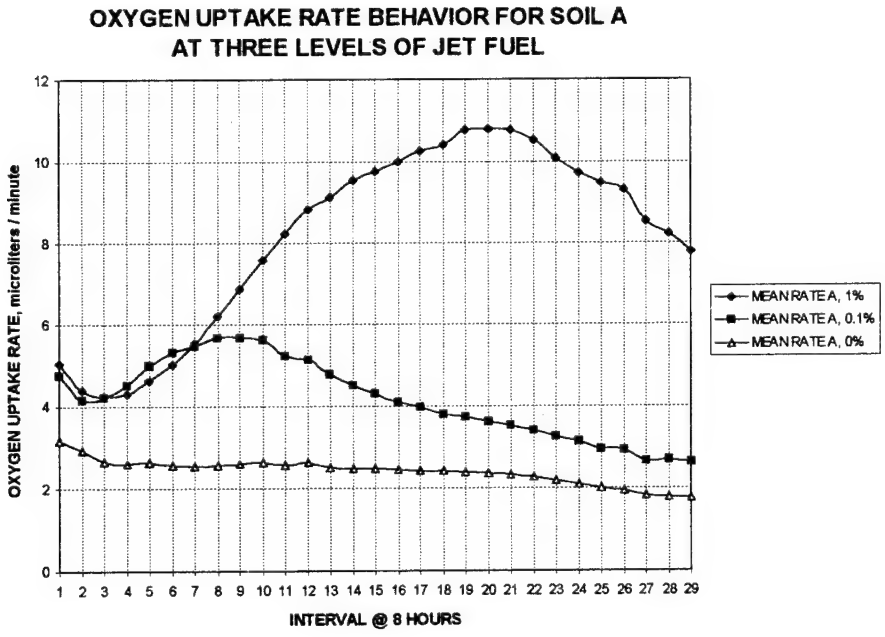



FIGURE 4.5 Respiration Curves for Soil A

The initial lag time for the 1% jet fuel treatment was about 8 hours longer than the 0.1% treatment. The slopes of respiration curves during the initial growth period were about equal; however, the 0.1% curve leveled off at its maximum rate almost four days before the 1% reached its maximum. The maximum rate of the 1% curve was over twice the maximum rate of the 0.1% curve. The slope of both treatments' declining respiration curves were approximately equal. The shapes of both these curves are overall very similar. The background respiration rate also demonstrated an initial lag time, but its respiration rate continued to fall throughout the experimental period.

Figure 4.6 shows the respiration rate curves for Soil B, the sandy, river bottom soil.

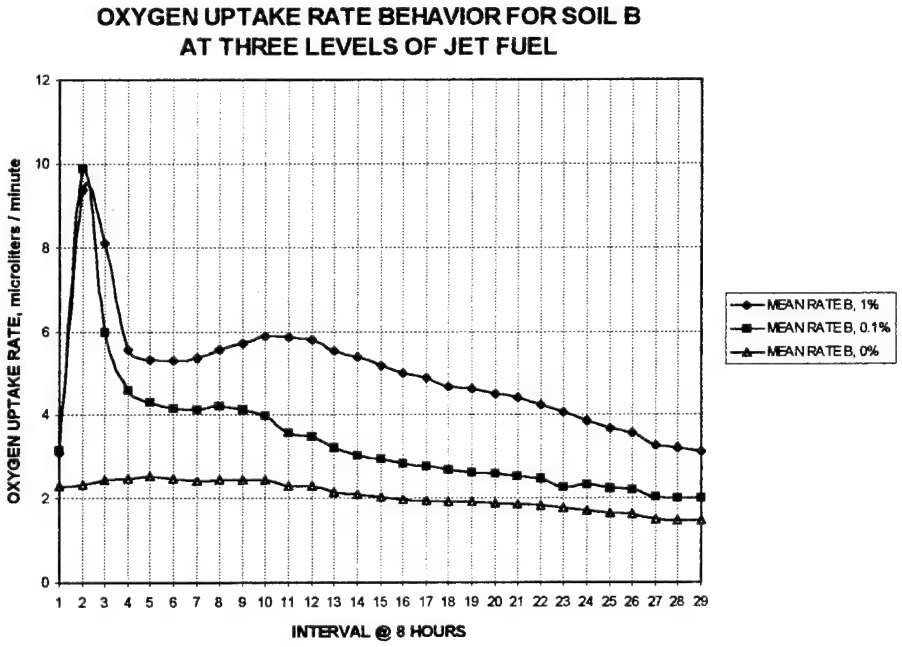



FIGURE 4.6 Respiration Curves for Soil B

Both treatments of Soil B showed the same rapid increase in respiration to their maximum rates during the first 8-hour period, followed by just as rapid a decrease. The maximum rates for both levels of jet fuel were remarkably similar as were the rates at which their respiration rapidly declined. Both curves indicated that a slight rebound in respiration occurred about one day following the rapid drop-off. The rebound lasted about half a day for the 0.1% treatment and about 1.5 days for the 1% treatment. Respiration then began slowing down, almost approaching background levels by the end of the experiment. The background rate showed a slight increase in respiration (again, with no time lag) followed by a steady decline for the rest of the observation period.

The rapid increase in respiration phenomenon that occurred during the first day by was investigated more closely by increasing the frequency of the sampling rate from one sample every 8 hours to one sample every 4 hours during the second run of the experiment. Figures 4.7 shows the results. The main fact that was revealed was a more accurate estimate of the maximum rates; they increased from about 10 μl/min to around 16 μl/min on average. The lag time of about 8 hours became evident; however, the increases and decreases in respiration were just as rapid.

Figure 4.8 shows the respiration rate curves for Soil C, the residential garden soil. Soil C demonstrated a similar pattern to Soil A; the 0.1% jet fuel curve was almost a scaled-down version of the 1% curve. An 8-hour time lag was followed by a rapid

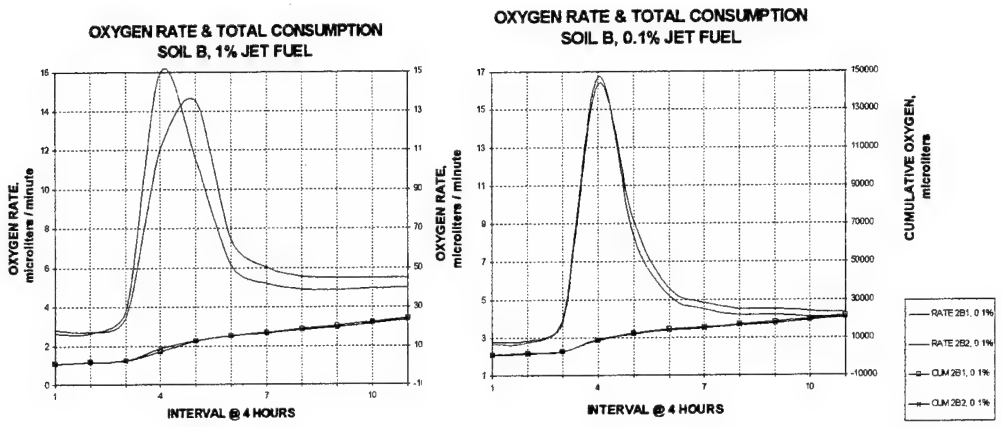



FIGURE 4.7 Four-hour Interval Curves for Soil B

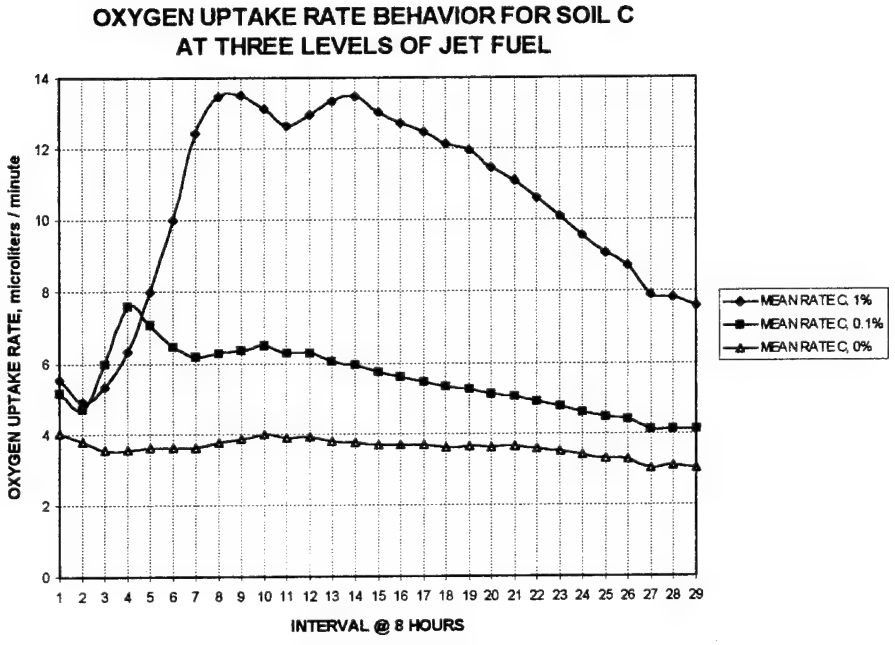



FIGURE 4.8 Respiration Curves for Soil C

increase in respiration up to the maximum rate. After reaching their maximum heights, both curves turned downward, for about a day, then exhibited a rebound in respiration. The 1% achieved approximately the same maximum respiration rate following the rebound where the 0.1% curve rebounded only slightly before the rate began its final descent.

### 4.1.3 Specific Biodegradation Kinetics

The 3/2 mineralization kinetics model described in Section 3.1.2.4 offered the best potential fit for a portion of the experimental data, although other models could also fit the curves. Using an empirical approach, the 95% confidence interval (CI) oxygen uptake curves from Soil A (most representative of an ideal respiration and growth pattern) were compared with the model. The experimental data showed several distinct stages which led to the use of this model. (Lag time was not considered in any of the models.) The increased response of the biological activity to increasing levels of jet fuel indicated a first order kinetics, included in the $\mathbf{k_2}$ linear growth term. All the response curves eventually leveled off and turned downward, indicating a limiting effect, independent of substrate concentration. The typical values used for the model's coefficients were: $k_0 = 1$, $k_1 = 0.01$, and $k_2 = 0.08$. In fact, a wide range and mix of these coefficients could be used to generate curves that fit within these same data. The model curves fit well within a portion of the 95% confidence interval envelopes of the experimental data. Figures 4.9 and 4.10 show the best attempts at these fits. The remaining 95% CI curves and an example of the model are contained in Appendix F.

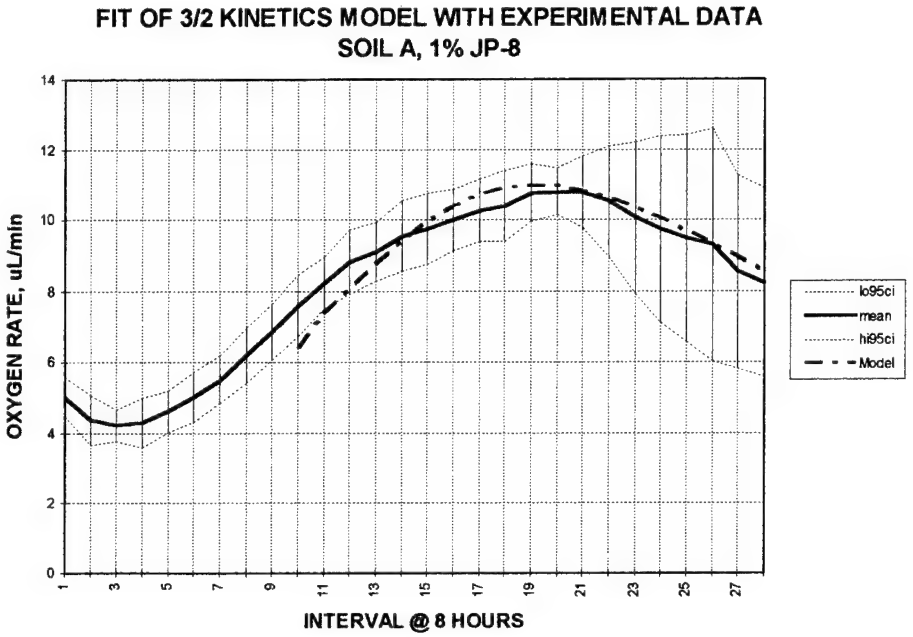



FIGURE 4.9 Fit of 3/2 Kinetics Model with Soil A, 1% JP8

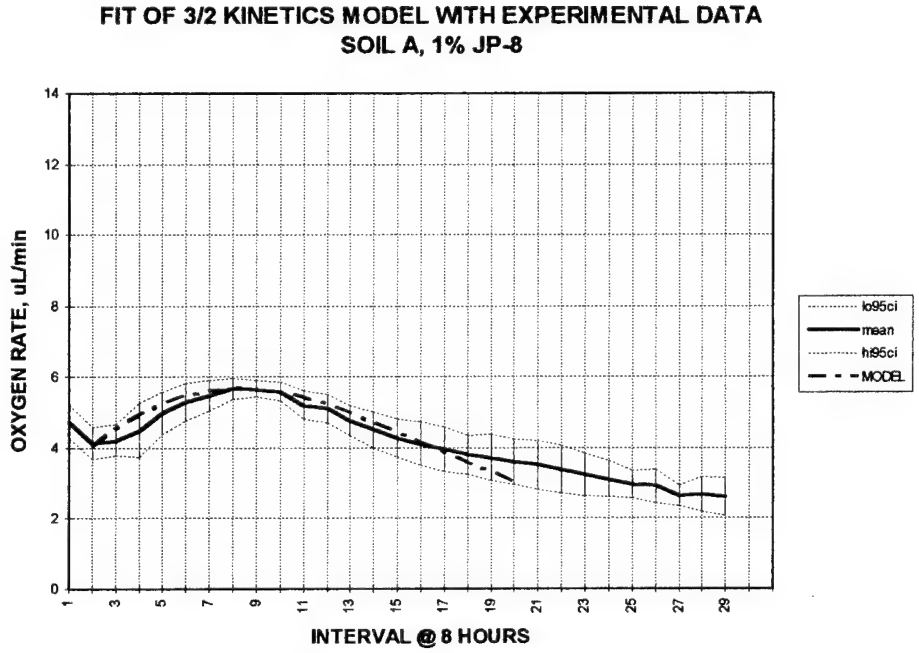



FIGURE 4.10 Fit of 3/2 Kinetics Model with Soil A, 0.1% JP8

### 4.1.4 Ratio of Oxygen Consumed to Carbon Dioxide Produced

The theoretical ratio of oxygen consumed to carbon dioxide produced for dodecane ($C_{12}H_{26}$) was 1.5417. The mean of the observed respiration ratio for all microcosms containing jet fuel and for the background soil respiration is given in Table 4.4.

TABLE 4.4

$O_2$:$CO_2$ RATIOS FOR HYDROCARBON AND BACKGROUND RESPIRATION

| | HYDROCARBON RESPIRATION | BACKGROUND RESPIRATION | EXPECTED RESPIRATION |
|---|---|---|---|
| MEAN | **3.0335** | **2.7918** | **1.5417** |
| STD. DEVIATION | **0.2337** | **0.1065** | - |
| MEDIAN | **2.9762** | **2.8100** | - |

The observed $O_2$:$CO_2$ ratio indicated there is still carbon and carbon dioxide to be accounted for. Assuming complete mineralization occurred and 25% of the carbon went to the synthesis of biomass, the ratio would be 2.0556 (Section 3.2.5). It is safe to assume that complete mineralization did not occur, because there was residual fuel in the soil at the end of the experiments. Therefore, the uptake of oxygen (together with the lack of carbon dioxide gas production) can also be attributed to the conversion of some of the carbon from the typical alkane (n = 12) into the many intermediate biodegradation products described in Section 3.2.5. Because mineralization was not complete, the carbon remained locked up in these intermediate compounds.

Table 4.5 shows the carbon dioxide accounting. Appendix G provides additional information about the ratios and the carbon dioxide. The $O_2$:$CO_2$ ratio of the background respiration was not significantly different than that of the hydrocarbon respiration.

Figures 4.11 through 4.14 show the distribution and normality of the two experimental data sets representing the $O_2$:$CO_2$ ratios of hydrocarbon and background respiration. Based on these data plots, it is reasonable to assume the data had a normal distribution about some mean values, and these two ratios (for background and hydrocarbon respiration) could be used with confidence to further describe any observed phenomena.

TABLE 4.5

RESPIRATION RATIO AND CARBON DIOXIDE ACCOUNTING

| | | Actual | Pred. | Pred. | Actual | Act. CO2 | Act. | Pred. CO2 | CO2 | CO2 |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment, | Soil Clay | Oxygen | O2:CO2 | Total | CO2 | Due to | O2:CO2 | Lost to | Unaccount | Unacct. |
| Soil, % JP8 | Content, % | Cons, ul | Ratio | CO2*, ul | Prod., ul | Biodeg., ul | Ratio** | Biomass*** | for, ul | % |
| C, 1 | 29 | 185221 | 1.5417 | 78542 | 56569 | 33563 | 3.6077 | 19635 | 25343 | 32.3 |
| C, 0.1 | 29 | 98426 | 1.5417 | 22244 | 33526 | 10521 | 3.2596 | 5561 | 6162 | 27.7 |
| C, 0 | 29 | 64133 | | | 23005 | | 2.7878 | | | |
| | | | | | | | | | | |
| A, 1 | 16 | 145246 | 1.5417 | 66398 | 47912 | 32690 | 3.1314 | 16599 | 17108 | 25.8 |
| A, 0.1 | 16 | 74015 | 1.5417 | 20195 | 24702 | 9480 | 3.2841 | 5049 | 5666 | 28.1 |
| A, 0 | 16 | 42881 | | | 15222 | | 2.8171 | | | |
| | | | | | | | | | | |
| B, 1 | 6 | 89355 | 1.5417 | 34231 | 31845 | 18841 | 2.801 | 8558 | 6832 | 20 |
| B, 0.1 | 6 | 60995 | 1.5417 | 15836 | 19778 | 6774 | 3.6044 | 3959 | 5104 | 32.2 |
| B, 0 | 6 | 36581 | | | 13004 | | 2.813 | | | |
| | | * = calculated from actual O2 produced (less background) | | | | | | | | |
| | | ** = CO2 adjusted for background respiration (0% JP-8 figures = ratio of background respiration) | | | | | | | | |
| | | *** = 25% of original carbon, from actual O2 produced (less background) | | | | | | | | |

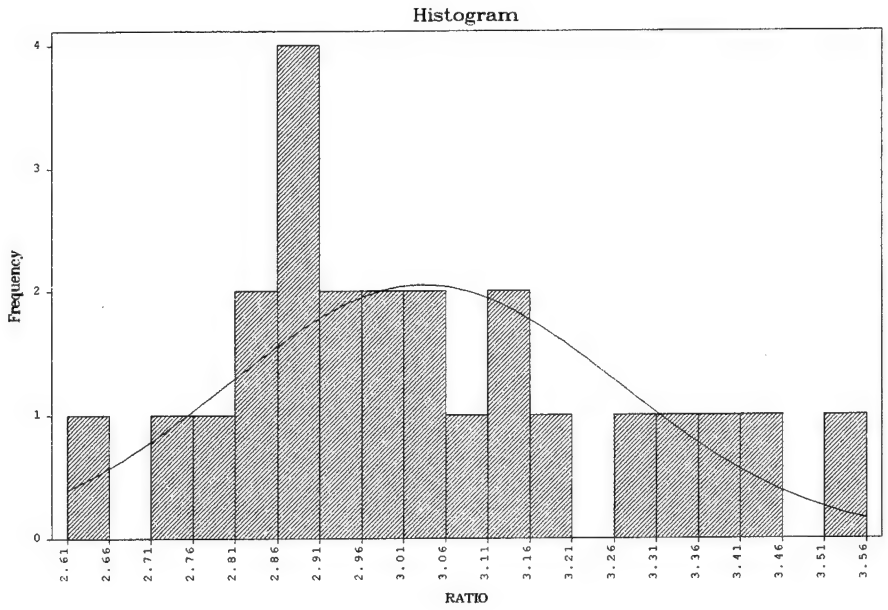



FIGURE 4.11 Histogram of Hydrocarbon Respiration Ratio Data

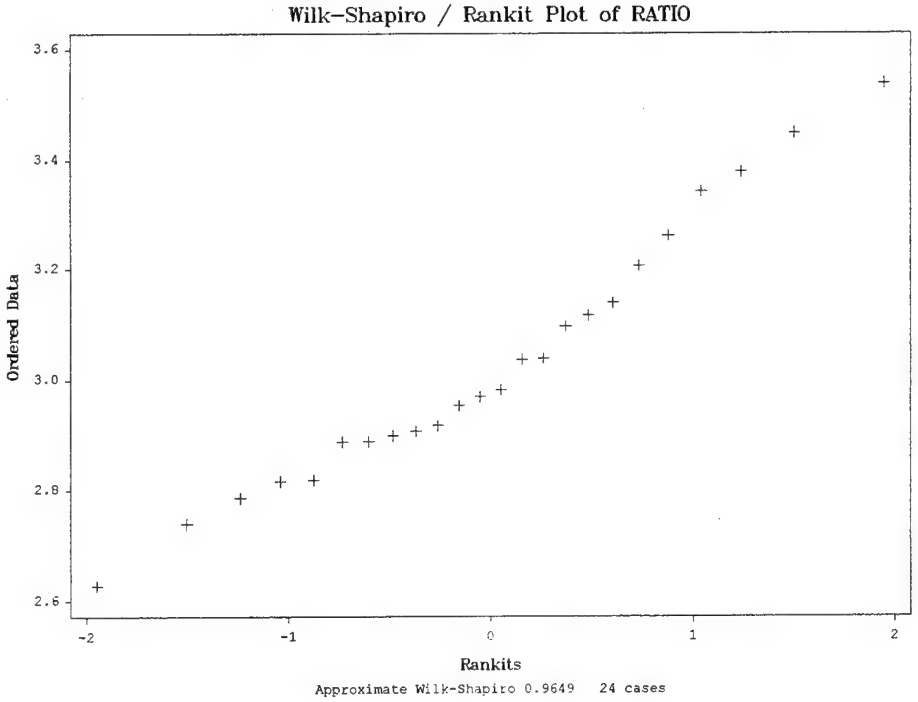



FIGURE 4.12 Test for Normality of Hydrocarbon Respiration Ratio Data

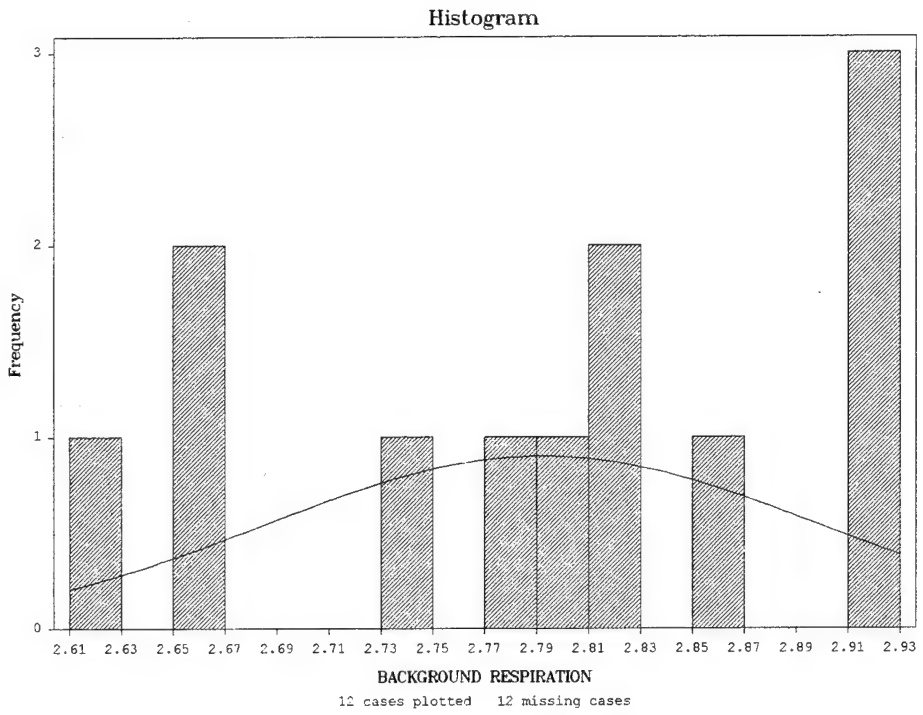



FIGURE 4.13 Histogram of Background Respiration Ratio Data

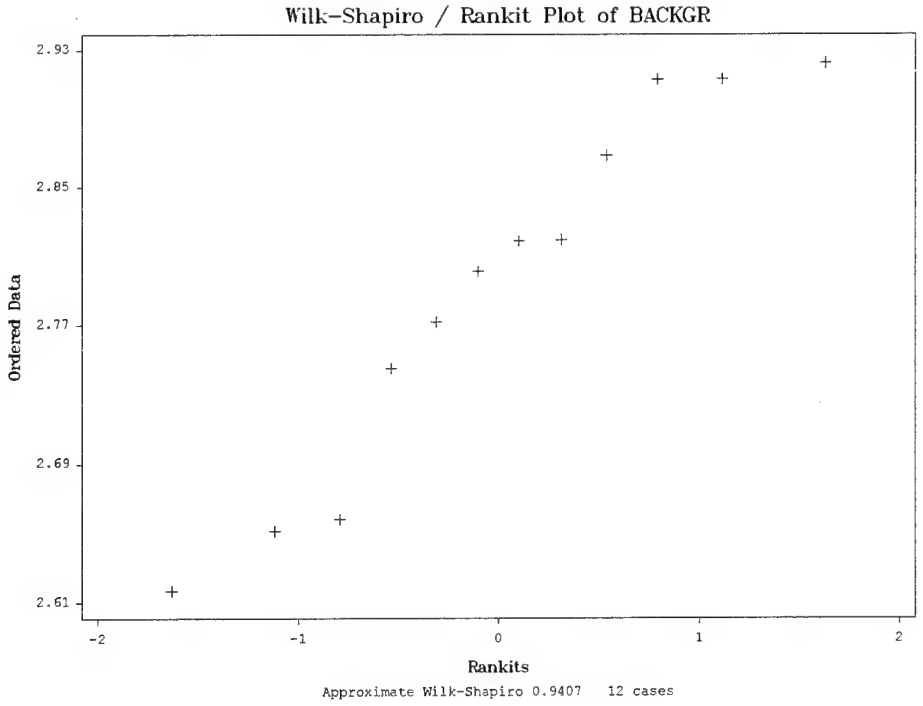



FIGURE 4.14 Test for Normality of Hydrocarbon Respiration Ratio Data

The figures demonstrate a reasonably normal distribution for both the background and hydrocarbon respiration ratios by the linear appearance of the Wilk-Shapiro / Rankit plots. The hydrocarbon respiration is more nearly normal than the background respiration because of its larger Wilk-Shapiro statistic (0.9649 versus 0.9407).

### 4.1.5 Nutrient Levels Affected by Fuel Levels

All three soils exhibited a strong negative correlation between fuel levels in the soil and nitrate levels remaining in the microcosms after the experimental runs. The low *p values* and significant $r^2$ values from the regression analyses lend confidence to these correlations. The 95% confidence intervals of these data were also quite small (especially Soil A), as shown on the regression plots in the following three figures.

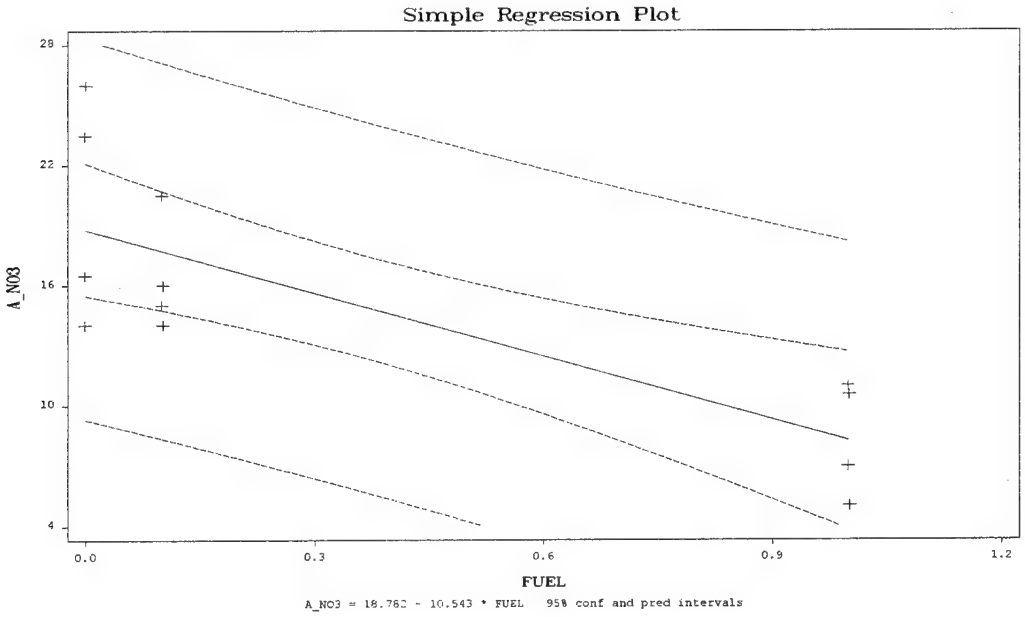



FIGURE 4.15 Regression Plot for Soil A: Nitrate vs. Fuel

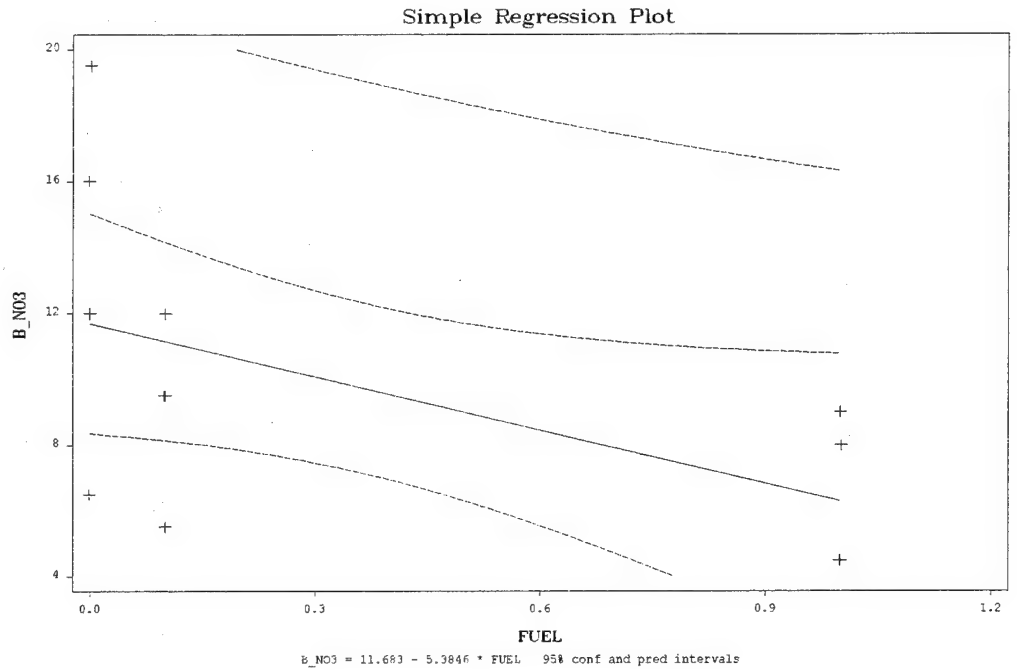



FIGURE 4.16 Regression Plot for Soil B: Nitrate vs. Fuel

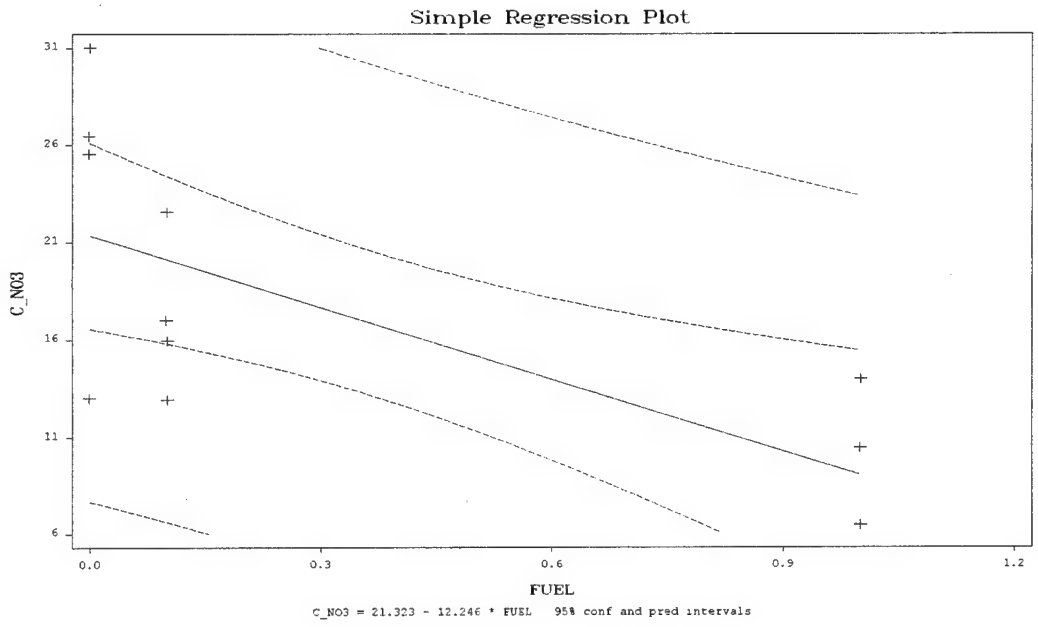



FIGURE 4.17 Regression Plot for Soil C: Nitrate vs. Fuel

Table 4.6 shows the models for the three soils' relationships between fuel and nitrates:

TABLE 4.6

REGRESSION MODELS FOR NITRATES

| SOIL | MODEL | STD. ERROR | COEFF. OF DETERMIN. | P VALUE $\alpha = .05$ |
|---|---|---|---|---|
| A | **$NO_3 = 18.543 - 10.543*FUEL$** | **s = 3.99** | **$r^2 = .6288$** | **SLOPE: .0021** |
| B | **$NO_3 = 11.683 - 5.846*FUEL$** | **s = 4.02** | **$r^2 = .3036$** | **SLOPE: .0633** |
| C | **$NO_3 = 21.323 - 12.246*FUEL$** | **s = 5.74** | **$r^2 = .5250$** | **SLOPE: .0077** |

Soils A and C demonstrate a significant inverse relationship between fuel level and nitrate levels by their *p values* much less than 0.05, the chosen level of significance. The coefficients of determination, however, indicate that only 53-63% of the variability of nitrate levels can be attributed to changes in fuel levels. The statistics for the Soil B model show an even more questionable relationship, to the point of accepting the null hypothesis that the slope is actually zero (**$p > 0.05$**). The relatively low value of **$r^2$** further decreases the significance of the relationship. Appendix H contains the data from the regression analysis that was done by the Statistix™ computer package as well as the raw data from the nutrient chemical analyses.

Table 4.7 shows that the relationships between fuel levels and phosphate levels for the soils were not so strong. The p values for the phosphate tests for correlation demonstrate this weak relationship.

TABLE 4.7

REGRESSION MODELS FOR PHOSPHATES

| SOIL | MODEL | STD. ERROR | COEFF. OF DETERMIN. | P VALUES $\alpha = .05$ |
|---|---|---|---|---|
| A | **$PO_4 = 55.6821 - 12.1786*FUEL$** | **s = 15.71** | **$r^2 = .1273$** | **SLOPE: .2550** |
| B | **$PO_4 = 97.4173 + 6.8846*FUEL$** | **s = 28.08** | **$r^2 = .0144$** | **SLOPE: .7105** |
| C | **$PO_4 = 122.079 - 35.9203*FUEL$** | **s = 46.60** | **$r^2 = .1260$** | **SLOPE: .2576** |

The following plots show a range from a slightly negative to slightly positive correlation. Problems encountered with the laboratory procedures may account for the inconsistent performance and weak correlations.

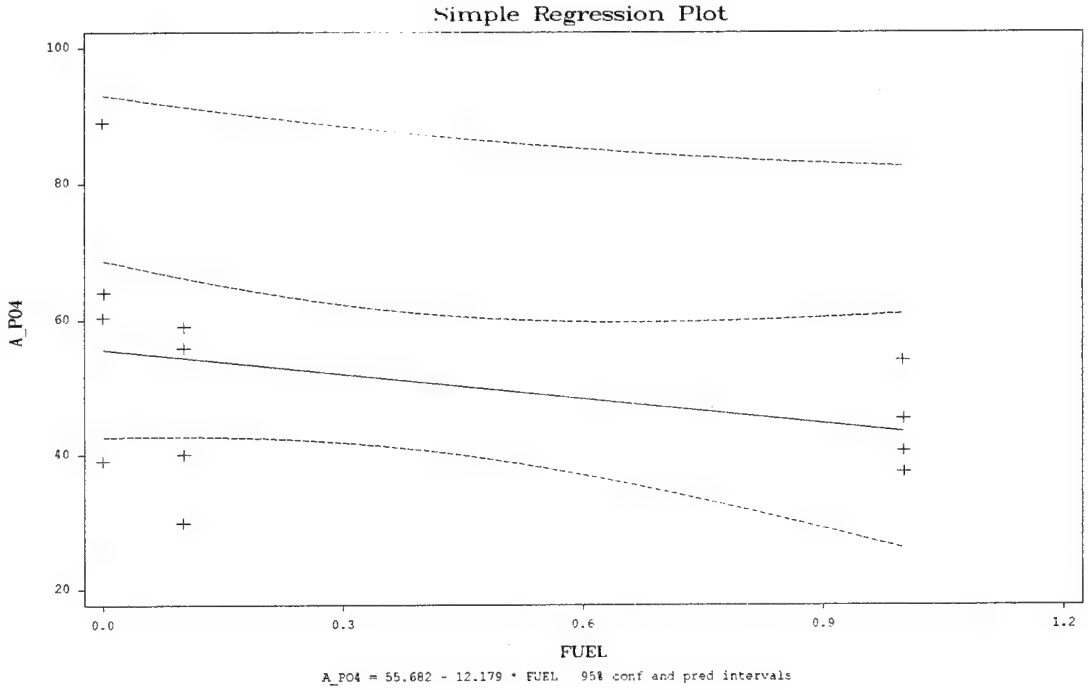



FIGURE 4.18 Regression Plot for Soil A: Phosphate vs. Fuel

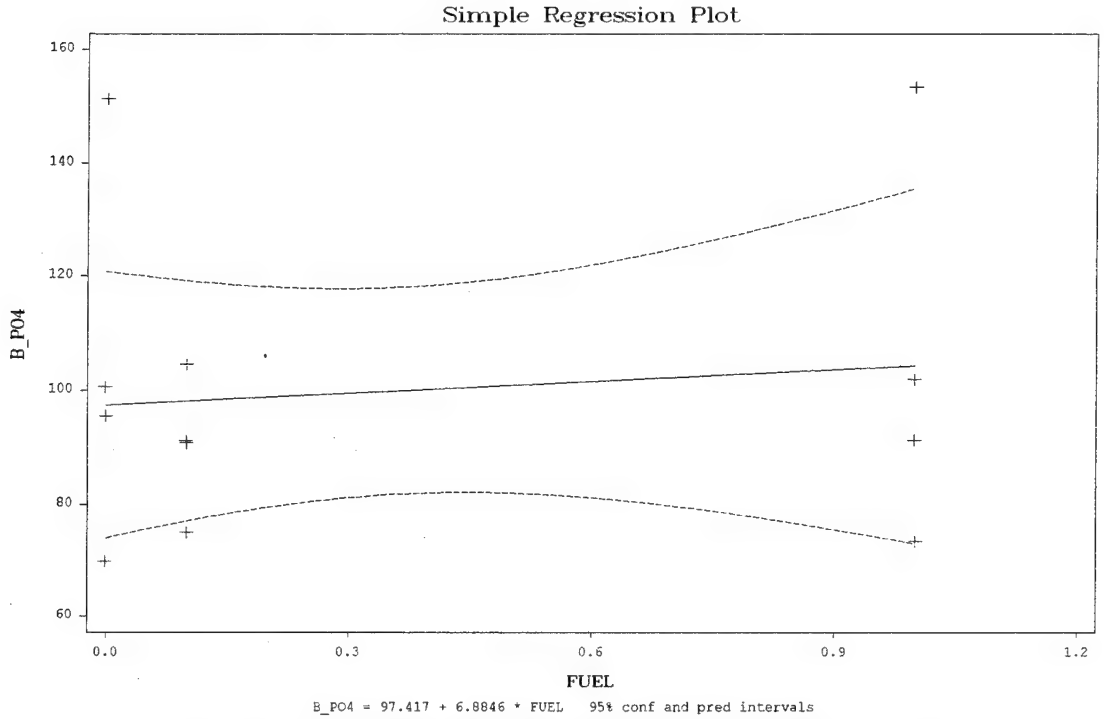



FIGURE 4.19 Regression Plot for Soil B: Phosphate vs. Fuel

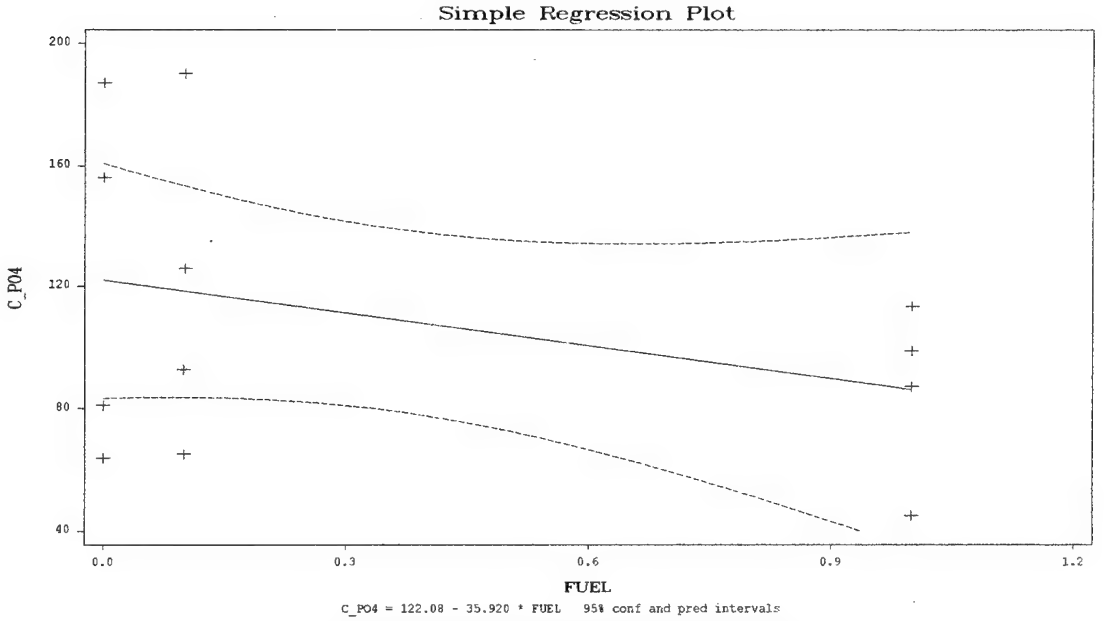



FIGURE 4.20 Regression Plot for Soil C: Phosphate vs. Fuel

### 4.1.6 Predicted Fuel Loss by Respirometer vs. Direct Measurement

The amount of fuel lost to biodegradation was predicted by the respirometer data and then measured directly by conducting a mass balance on the fuel in the soil. The respirometer predictions are in Table 4.8.

TABLE 4.8

FUEL CONSUMED AS CALCULATED FROM RESPIROMETER OUTPUT

| SOIL TYPE | CLAY (%) | FUEL ADDED (microliters) | FUEL CONSUMED (microliters) |
|---|---|---|---|
| A | **16** | **1266.7** | **48.3** |
| A | **16** | **126.7** | **14.7** |
| B | **6** | **1253.7** | **24.9** |
| B | **6** | **125.4** | **11.5** |
| C | **29** | **1372.6** | **57.2** |
| C | **29** | **137.3** | **16.2** |

The higher levels of fuel always produced the higher levels of consumption and the higher levels of clay also produced the higher levels of consumption. This was also demonstrated earlier in Section 4.1.1, using total oxygen consumption, which can be directly equated to total fuel consumption.

A mass balance was built around the original, known amount of jet fuel that was added to each microcosm at the beginning of each run. The two components that were measured after the run were the fuel lost to evaporation and the fuel remaining in the soil. The difference between the sum of these and the original jet fuel added to the microcosms is

the amount of fuel lost to biodegradation. Table 4.9 shows the amount of fuel consumed by biodegradation, measured directly by the mass balance of fuel originally placed in the microcosms.

TABLE 4.9

MASS BALANCE OF JET FUEL IN MICROCOSMS

| SOIL TYPE | CLAY (%) | ORIG. FUEL (%) | ORIG. FUEL (µl) | LOST TO EVAP. (µl) | REMAINING IN SOIL (µl) | LOST TO BIODEG. (µl) |
|---|---|---|---|---|---|---|
| A | **16** | **1** | **1,266.7** | **323.5** | * | * |
| A | **16** | **0.1** | **126.7** | **70.1** | * | * |
| B | **6** | **1** | **1,253.7** | **267.1** | * | * |
| B | **6** | **0.1** | **125.4** | **30.8** | * | * |
| C | **29** | **1** | **1372.6** | **316.5** | * | * |
| C | **29** | **0.1** | **137.3** | **31.4** | * | * |

* - data not available

The data representing the amount of jet fuel remaining in the soil was not available or was inconclusive at the time of publication. Had this component of the mass balance been available, the amount of jet fuel lost to bioremediation could have been calculated. Then the two independent methods--the theoretical prediction of the respirometer (Table 4.8) and the direct measurement of soil and volatilized components--could have been evaluated by a paired t test to determine the statistical significance of the difference between the two methods. This last research element was not completed.

Observations were made about the evaporation component, however. Fuel evaporation varied with jet fuel level and soil type. Soil B lost no detectable fuel to evaporation at

the 0.1% jet fuel level during the first run of the experiment and then lost an amount similar to the other soils during the second run. Otherwise, the soils were remarkably similar in their volumes of fuel lost to evaporation. (The high levels for Soils A and C in the third "1%" set are most likely an anomaly of the test method.) The percent of initial fuel lost to evaporation is very different, however. Soil C remains fairly constant in percent lost data, where Soils A and B show marked decreases at the higher fuel levels.

Figure 4.21 shows the fuel losses to evaporation graphically, first, as the volume of fuel (the bars on the graph) and then as percentage of the original fuel that was added to the microcosms (the lines). Appendix I contains representative TGA data.

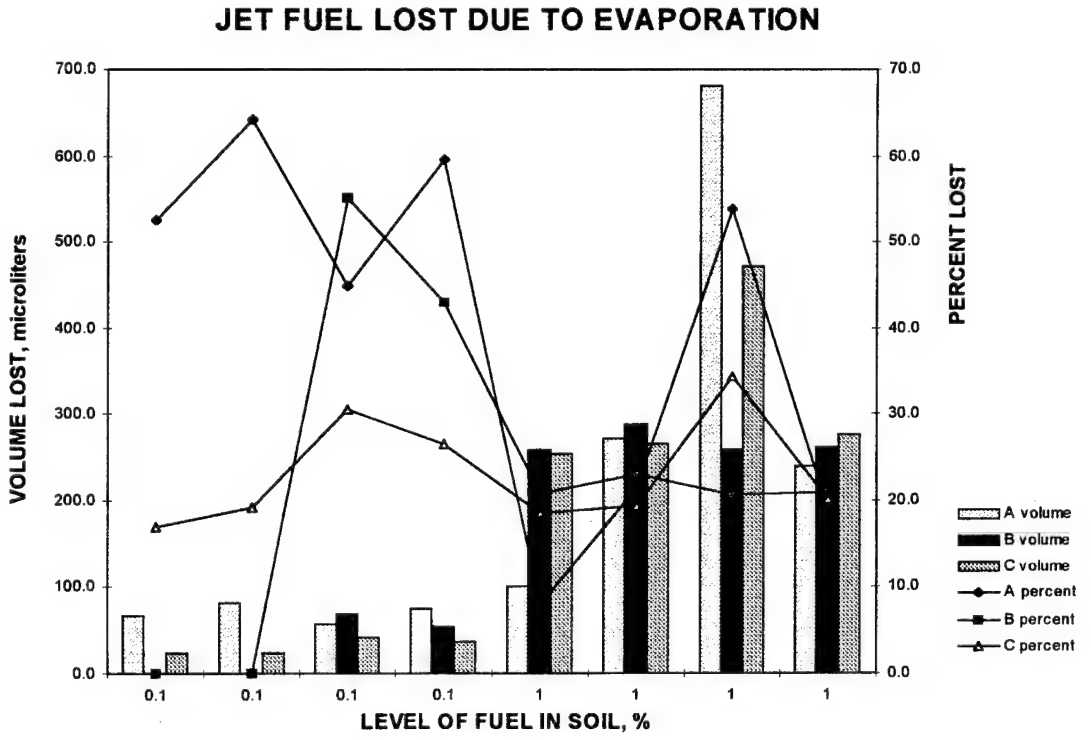



FIGURE 4.21 Fuel Losses to Evaporation

## 4.2 Sources of Error

There were many potential sources of error that could have affected this experiment. The respirometer, although it contains elaborate physical and computational systems to minimize error, remains a complex piece of equipment. Leakage of air from the system during the experiment was not tested except at the beginning, and it could introduce major errors in the gas measurements. Because they were natural products, the soils could be highly variable, in terms of their physical, chemical, and biological constituents, especially given the relatively small sample sizes used. The laboratory tests were performed according to the manufacturer's instructions, however, less-than-ideal laboratory conditions may have introduced some errors from improper glassware cleaning, lack of proper temperature controls, and the unknown quality of the distilled water used, which was purchased from a local market.

## 4.3 Limitations of Experimental Design

The primary limitations were the limited number of factors examined (two) and the low number of factor levels (three each). There were also many more factors, mainly soil chemical constituents such as heavy metals, trace organics, and so on, that were not fully quantified that may have influenced the responses. Also, no attempt was made to identify or quantify the primary biological agents responsible for the biodegradation. The various metabolic pathways the biodegradation could have taken could not be identified without these pieces. Only one level of soil moisture was examined and this could also significantly affect the potential for biodegradation. The soil constituents that were

examined were measured only at the beginning and end of the experimental run. More information could have been obtained from the same experiment if periodic soil samples were taken from the microcosms as the run progressed. There was opportunity for this when the respirometer apparatus was periodically stopped to change out the moisture absorbing tubes from the microcosms.

# V. Conclusions and Recommendations

## 5.1 Conclusions

Increasing levels of jet fuel in the three soils tested in this experiment produced increasing amounts of biological activity, as measured by the oxygen uptake. This indicated that conditions in all three soils were adequate to support biodegradation and that the microbial populations present were initially limited by the lack of carbon sources.

Increasing levels of clay in the three soils produced increasing levels of biological activity, especially at the highest level of fuel. There could be many explanations for this observation; however, the most probable was the increased surface area provided by the clay offered more area for the biological activity to take place.

It could also be concluded that increasing levels of sand in the three soils produced decreasing levels of biological activity due to the lack of surface area available for microbial activity.

There was no correlation between the oxygen uptake and the organic carbon content of the soils, therefore it is difficult to say what role adsorption played, if any, in this effect.

The two soils with the most clay demonstrated similar biodegradation characteristics. The both exhibited an observable lag time (acclimation), a slowly increasing oxygen uptake rate (substrate utilization), followed by a leveling out (some factor reaching a limiting level) and finally, growth at a decreasing rate (decay). These events each took typically one to two days to accomplish--longer with increasing levels of fuel. This was attributed to the time it took for the substrate and microbes to reach each other due to the extremely small interstitial nature of the clay soil structure. The sandy soil exhibited an almost instantaneous response to the fuel, indicating that the microbial population and substrate came together much faster.

The observed kinetics were a complex combination, which led to the 3/2 model, which provided a combination of factors, including zero (high concentration of fuel versus microorganisms, which describe the initial conditions), and perhaps even Michaelis-Menten (the transition from state where rate of decomposition changes from being proportional to being independent of fuel concentration). It would be difficult to find one single model that could describe in total, what was observed. In natural ecosystems, a variety of factors can alter the shapes of the substrate reducing curves: predation by protozoa, time for the organisms to build up, toxin accumulation, depletion of nutrients, presence of other substrates that repress utilization of the compound of interest, binding of compound to colloidal matter, such as clay. (Riser-Roberts, p32)

The observed ratio of oxygen to carbon dioxide was much greater than expected, indicating there was a carbon fraction unaccounted for. The initial assumptions used in this research did not include loss of carbon to biomass from 25 - 40% of the original carbon) nor did it include something other than complete mineralization. Adjusting for biomass did not make up for the difference. This leads to two conclusions: 1) incomplete mineralization left some carbon tied up in alcohols and other intermediate hydrocarbon compounds and 2) significant carbon dioxide dissolved in soil moisture, forming carbonic acid, which then reacted with soil minerals to form carbonates and bicarbonates. The first has to do with the biodegradation reactions; the second with the disappearance of carbon dioxide gas, once it was formed.

There was a strong inverse correlation between the initial level of fuel in the soil and the level of nitrates left in the soil after fourteen days in the microcosms. Microcosms with higher levels of fuel showed significantly smaller amounts of nitrates remaining in the soil (over the background soil respiration) than the lower level of fuel. This is a strong indication that the soil microbes consumed more of this nutrient while biodegrading the larger quantity of fuel.

A much weaker relationship was observed with the phosphates; however, a consistent error in the laboratory procedure for phosphates (an incorrect extraction technique was used) could explain this lack of correlation. It was expected that phosphate reduction due to microbial activity would be just as observable as the nitrate reductions.

It was impossible to compare the estimate of total fuel biodegraded based on oxygen consumption to an estimate using direct measurements because the data describing the amount of fuel left in the soils after fourteen days in the microcosms was incomplete. A complete mass balance on the fuel could not be determined.

Fuel evaporation observations showed that the higher the initial level of fuel, the more fuel was lost to evaporation, both absolutely and proportionally. The fuel volatilizes continually from the soil while in the microcosms and gets carried into the organic vapor traps during the respirometer's sampling cycles. The higher the initial concentration, the more the fuel could spread out onto the soil particles and therefore more area was available for evaporation.

### 5.2 Improvements

This experiment could have been improved by freezing the bulk soil samples, rather than refrigerating them. The soil nutrients changed during the time the soils were in storage, making the nutrient losses more difficult to assess.

Levels of nutrients should have been measured in the "t = 0" soils, that is in the soils in each of the microcosms at the beginning of each experimental run. This would have produced better nutrient consumption data, measuring the actual loss of nutrients due to fuel, rather than the difference over the background microcosms.

More complete soil chemistry, specifically initial and final pH, bicarbonates and carbonates, may have revealed the missing carbon dioxide.

### 5.3 Follow-On Research

Increase the number of levels for each factor. One or two more different soils may further explain the effect of clay and reveal the effect of soil organic carbon. Additional levels of jet fuel may more clearly demonstrate the kinetics at work. This would mean more individual experimental runs because the respirometer can monitor only twenty channels at a time in its present configuration.

Sample the microcosms immediately following any notable events, such as the peak rates in Soil B and the rebounding rates in Soil C. Reasons for these behaviors may be uncovered by measuring the nutrient levels.

Design experiments to rigorously investigate and identify mineralization kinetics using respirometer data. Determine if there is any "best" kinetic model for a specific soil type.

### 5.4 Summary

Soils contaminated with spilled jet fuel JP-8 are readily biodegraded under aerobic conditions by indigenous soil microorganisms. The Air Force's changeover to JP-8 will not preclude the use of intrinsic biodegradation as a means of remediating soils contaminated by spills of this new fuel.

Intrinsic biodegradation of jet fuel contaminated soils is expected to be more effective in soils with higher clay content or lower sand content. These soil constituents should be evaluated when designing intrinsic bioremediation as a treatment option for jet fuel contaminated soils.

Respirometry offers a simple means to evaluate many aspects of the biodegradation of jet fuel contaminated soils. The capacity of a natural soil for intrinsic bioremediation of jet fuel contamination can be quantified within a relatively short time, for example.

## Appendix A: Additional Jet Fuel JP-8 Characteristics

Jet fuel JP-8 is defined in Military Specification MIL-T-83133D, 29 January 1992. It is classified as an aviation turbine fuel with additives that provide corrosion inhibition, lubricity improvement, and fuel system icing inhibition. It is a kerosene type hydrocarbon distillate fuel refined from crude oil feed stocks derived from petroleum, tar sands, oil shales, or their mixtures. The distribution of hydrocarbon species is shown in Figure 3.6. Approved antioxidant chemicals, such as 2,6-di-tert-butyl-4-methylphenol, are added at the rate of 17.2-24.0 mg/l of fuel. A metal deactivator, such as N,N'-disalicylidene-1,2-propanediamine, is blended into the fuel up to the amount 5.8 mg/l. Static dissipaters, corrosion inhibitors, and fuel system icing inhibitors are described by other military specifications and added accordingly. Table A.1 provides some other selected characteristics that may be of interest for this research.

TABLE A.1
SELECTED SPECIFICATIONS OF JP-8

| PROPERTY | MIN | MAX | ASTM STD |
|---|---|---|---|
| Aromatics, vol % | - | 25.0 | D1319 |
| Olefins, vol % | - | 5.0 | D1319 |
| Sulfur Mercaptan, mass % | - | 0.002 | D3227 |
| Flash Point, °C | 38 | - | D93, D3828 |
| Density, kg/L @15°C | 0.775 | 0.840 | D1298, D4052 |
| Freezing Point, °C | - | -47 | D2386 |
| Viscosity, centistokes @ -20°C | - | 8.0 | D445 |
| Hydrogen Content, mass % | 13.4 | - | D3701, D3343 |
| Particulate Matter, mg/L | - | .1.0 | D2276 |
| Fuel System Icing Inhibitor, vol % | 0.10 | 0.15 | D5006 |
| Fuel Electrical Conductivity, pS/m | 150 | 600 | D2624 |

## Appendix B: Soil Characterization Report

A private civil engineering laboratory was contracted to provide limited physical and chemical analyses on the three soils used in this experiment. Industry standard laboratory methods and tests were requested and the methods and quality control information were provided with the results. The following pages contain the complete laboratory reports on the soils.

## Particle-Size Analysis
## ASTM D-422

| | | | | | |
|---|---|---|---|---|---|
| Client: | Jim Baker | Sample # | A | Date: | 06/22/95 |
| Project: | Thesis | | | Tech: | P.W. |
| | | | | Depth: | |
| Project # | 95050791 | | | Assumed Gs: | 2.70 |

| | | | | | |
|---|---|---|---|---|---|
| Total Sample Weight = | 98.02 | grams | Hydrometer Sample Weight = | 35.94 | grams |

| Sieve Sizes | Weight Retained | % Retained | % Passing |
|---|---|---|---|
| 1" | 0.0 | 0.0 | 100.0 |
| 3/4" | 0.0 | 0.0 | 100.0 |
| 3/8" | 1.4 | 1.4 | 98.6 |
| #4 | 2.8 | 2.9 | 97.1 |
| #10 | 7.5 | 7.7 | 92.3 |
| #40 | 20.3 | 20.7 | 79.3 |
| #200 | 10.7 | 44.3 | 55.7 |

| Elapsed Time (min) | Hydro Reading | Temp. Correct. Value | Corrected Hydro Reading | % Total in Susp. | K | Effective Length (cm) | Particle Diameter (mm) |
|---|---|---|---|---|---|---|---|
| 2 | 25.0 | 5.5 | 19.5 | 42.60 | 0.01328 | 13.11 | 0.0340 |
| 15 | 18.0 | 5.5 | 12.5 | 27.30 | 0.01328 | 14.26 | 0.0129 |
| 60 | 14.0 | 5.5 | 8.5 | 18.57 | 0.01328 | 14.91 | 0.0066 |
| 240 | 12.0 | 5.5 | 6.5 | 14.20 | 0.01328 | 15.24 | 0.0033 |
| 1440 | 11.0 | 5.5 | 5.5 | 12.01 | 0.01328 | 15.4 | 0.0014 |

**Summary of Grain Size Distribution**

| | |
|---|---|
| 3 | % GRAVEL |
| 5 | % COARSE SAND |
| 13 | % MEDIUM SAND |
| 24 | % FINE SAND |
| 39 | % SILT |
| 16 | % CLAY (<0.005mm) |

**Atterberg Limits**

| | |
|---|---|
| Liquid Limit | NP |
| Plastic Limit | NP |
| Plasticity Index | NP |

**Natural Moisture Content**
25.3%

**Unified Soil Classification System**

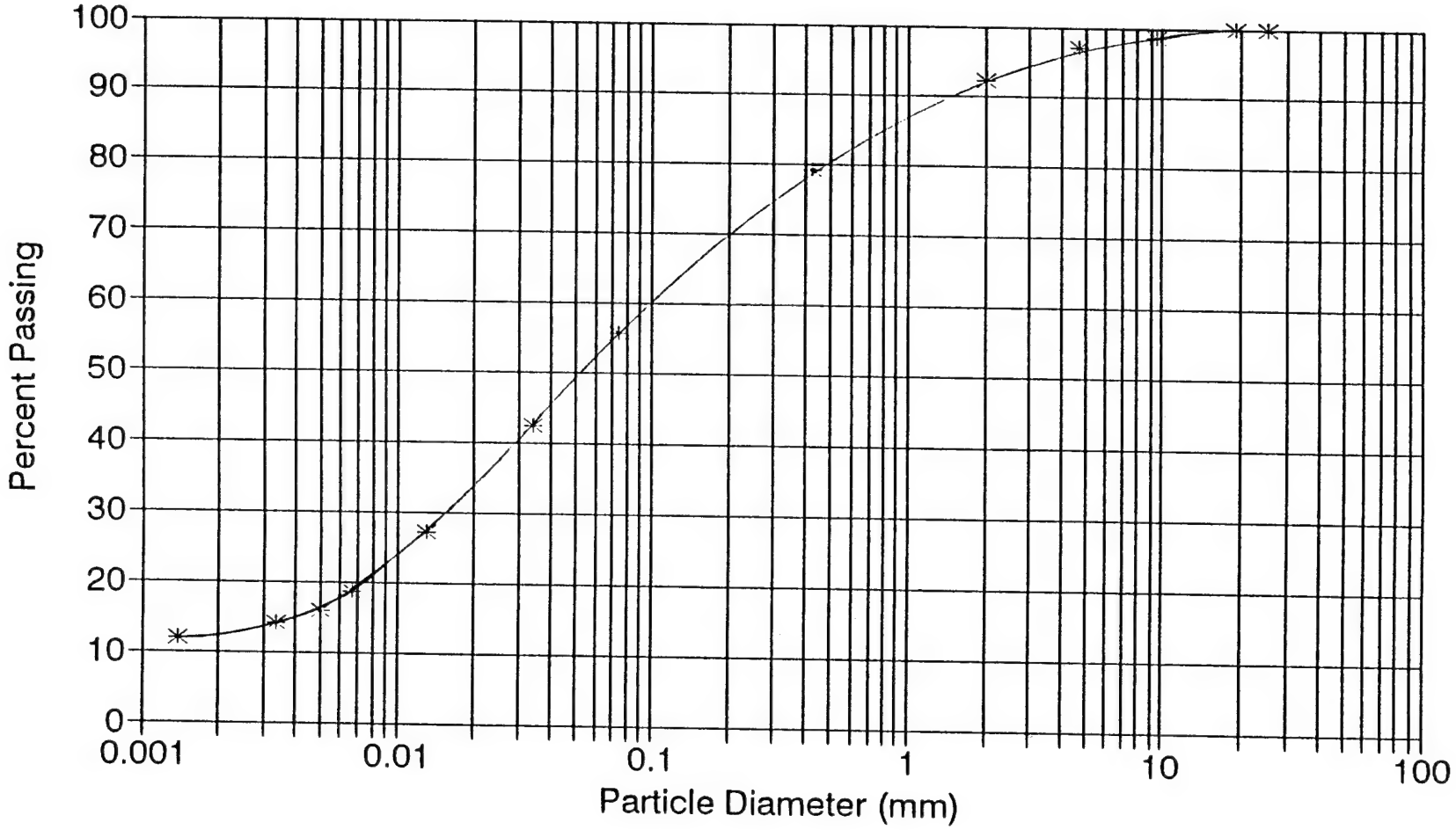
| | |
|---|---|
| Group Name: | Sandy Silt |
| Group Symbol: | ML |



GTL ENGINEERING

# Particle-Size Distribution
ASTM-D422

Jim Baker: Sample No. A




CTL ENGINEERING

GTL Engineering Inc.
2860 Fisher Road, P.O. Box 44469, Columbus, Ohio 43204
Phone: 614/276-8123 • Fax: 614/276-6377



# Report on Sample of Soil

Project No.: 95050791

July 12, 1995

Client: Jim Baker
3275 Boxwood Drive
Fairborn, Ohio 45324

Lab ID No.: 95-30656

Identification: One soil sample submitted 6-20-95, identified as A.

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TEST METHODS: pH by Method 9040 in USEPA Doc. SW 846, Total Organic Carbon by Combustion, Phosphate by Method 365.3, and Ammonia by Method 350.3, both from USEPA Doc. EPA-600/4-79-020, Nitrate by Ion Selective Electrode Potentiometry.

TEST RESULTS:

| Parameter | Result |
|---|---|
| pH | 7.92 S.U. |
| Total Organic Carbon | 7.04 % |
| Phosphate | 2.80 mg/kg |
| Ammonia as Nitrogen | 20.3 mg/kg |
| Nitrate as Nitrogen | 280 mg/kg |

QUALITY CONTROL DATA:

| Sample ID | Parameter | Reproducibility | Spike Recovery |
|---|---|---|---|
| 95-30656 A | pH | 100 % | -- |
| 95-30656 A | TOC | 103 % | -- |
| 95-30656 A | Phosphate | -- | 99 % |
| 95-30656 A | Ammonia | -- | 106 % |
| 95-30656 A | Nitrate | 98 % | -- |

---

Respectfully submitted,

Richard Herrold, Chemist

## Particle-Size Analysis
## ASTM D-422

| | | | | | |
|---|---|---|---|---|---|
| Client: | Jim Baker | Sample # | B | Date: | 06/22/95 |
| Project: | Thesis | | | Tech: | P.W. |
| | | | | Depth: | |
| Project # | 95050791 | | | Assumed Gs: | 2.68 |

| | | | | | |
|---|---|---|---|---|---|
| Total Sample Weight = | 118.40 | grams | Hydrometer Sample Weight = | 63.73 | grams |

| Sieve Sizes | Weight Retained | % Retained | % Passing |
|---|---|---|---|
| 1" | 0.0 | 0.0 | 100.0 |
| 3/4" | 0.0 | 0.0 | 100.0 |
| 3/8" | 0.0 | 0.0 | 100.0 |
| #4 | 0.0 | 0.0 | 100.0 |
| #10 | 0.3 | 0.2 | 99.8 |
| #40 | 47.8 | 40.4 | 59.6 |
| #200 | 47.7 | 85.0 | 15.0 |

| Elapsed Time (min) | Hydro Reading | Temp. Correct. Value | Corrected Hydro Reading | % Total in Susp. | K | Effective Length (cm) | Particle Diameter (mm) |
|---|---|---|---|---|---|---|---|
| 2 | 18.0 | 5.5 | 12.5 | 11.62 | 0.01336 | 14.26 | 0.0357 |
| 15 | 14.0 | 5.5 | 8.5 | 7.90 | 0.01336 | 14.91 | 0.0133 |
| 60 | 12.0 | 5.5 | 6.5 | 6.04 | 0.01336 | 15.24 | 0.0067 |
| 240 | 11.0 | 5.5 | 5.5 | 5.11 | 0.01336 | 15.4 | 0.0034 |
| 1440 | 10.0 | 5.5 | 4.5 | 4.18 | 0.01336 | 15.56 | 0.0014 |

**Summary of Grain Size Distribution**

| | |
|---|---|
| 0 | % GRAVEL |
| 0 | % COARSE SAND |
| 40 | % MEDIUM SAND |
| 45 | % FINE SAND |
| 9 | % SILT |
| 6 | % CLAY (<0.005mm) |

**Atterberg Limits**

| | |
|---|---|
| Liquid Limit | NP |
| Plastic Limit | NP |
| Plasticity Index | NP |

**Natural Moisture Content**
18.3%

**Unified Soil Classification System**

| | |
|---|---|
| Group Name: | Silty Sand |
| Group Symbol: | SM |



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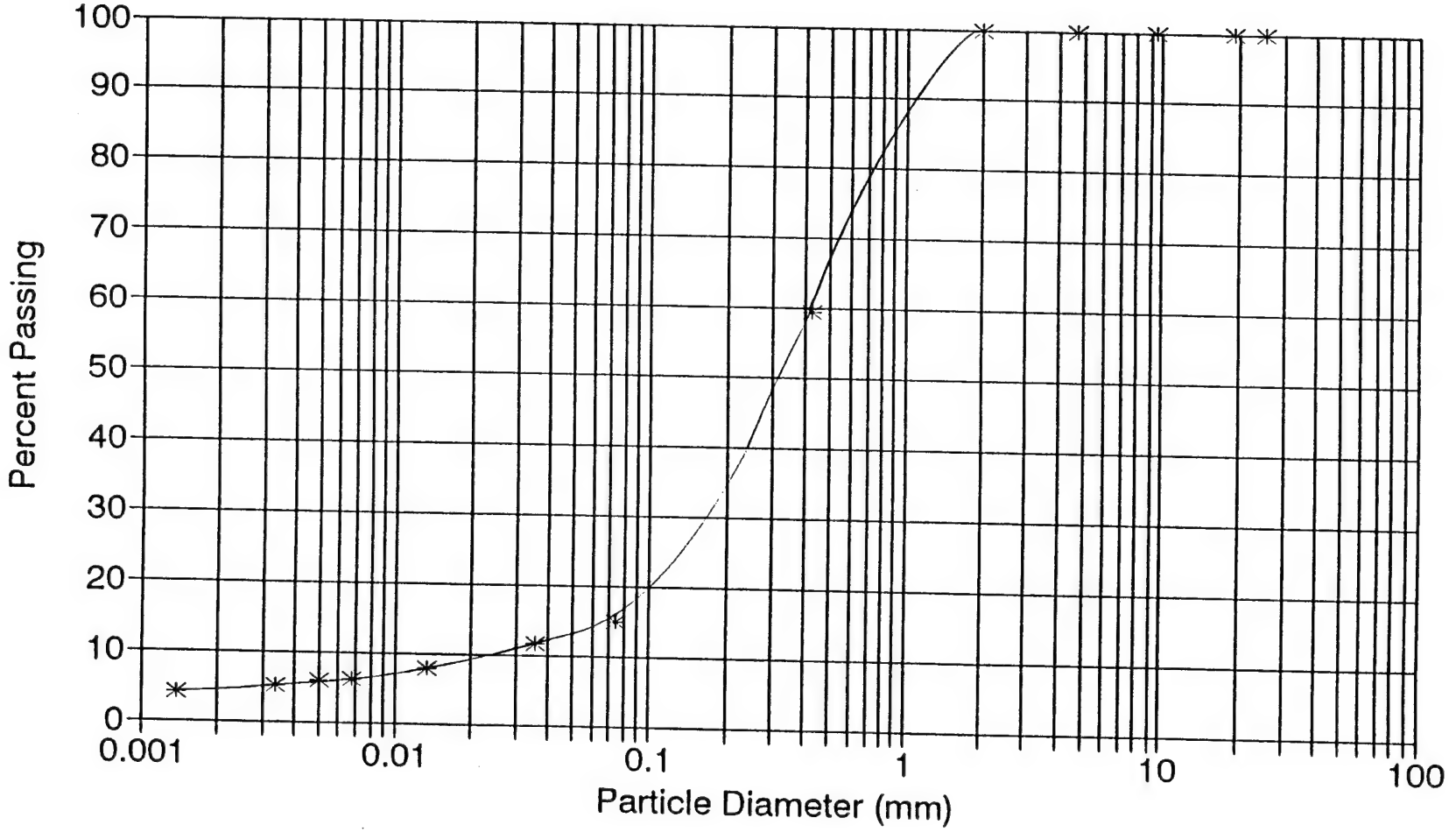
# Particle-Size Distribution
ASTM-D422

Jim Baker: Sample No. B

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*Established in 1927.*

# Report on Sample of Soil

Project No.: 95050791

July 12, 1995

Client: Jim Baker
3275 Boxwood Drive
Fairborn, Ohio 45324

Lab ID No.: 95-30656

Identification: One soil sample submitted 6-20-95, identified as B.

---

TEST METHODS: pH by Method 9040 in USEPA Doc. SW 846, Total Organic Carbon by Combustion, Phosphate by Method 365.3, and Ammonia by Method 350.3, both from USEPA Doc. EPA-600/4-79-020, Nitrate by Ion Selective Electrode Potentiometry.

TEST RESULTS:

| Parameter | Result |
|---|---|
| pH | 8.08 S.U. |
| Total Organic Carbon | 2.05 % |
| Phosphate | 2.60 mg/kg |
| Ammonia as Nitrogen | 11.7 mg/kg |
| Nitrate as Nitrogen | 336 mg/kg |

QUALITY CONTROL DATA:

| Sample ID | Parameter | Reproducibility | Spike Recovery |
|---|---|---|---|
| 95-30656 A | pH | 100 % | -- |
| 95-30656 A | TOC | 103 % | -- |
| 95-30656 A | Phosphate | -- | 99 % |
| 95-30656 A | Ammonia | -- | 106 % |
| 95-30656 A | Nitrate | 98 % | -- |

---

Respectfully submitted,

Richard Herrold, Chemist

## Particle-Size Analysis
## ASTM D-422

| | | | | | |
|---|---|---|---|---|---|
| Client: | Jim Baker | Sample # | C | Date: | 06/22/95 |
| Project: | Thesis | | | Tech: | P.W. |
| | | | | Depth: | |
| Project # | 95050791 | | | Assumed Gs: | 2.72 |

| Total Sample Weight = | 105.43 grams | Hydrometer Sample Weight = | 40.87 grams |
|---|---|---|---|

| Sieve Sizes | Weight Retained | % Retained | % Passing |
|---|---|---|---|
| 1" | 0.0 | 0.0 | 100.0 |
| 3/4" | 0.0 | 0.0 | 100.0 |
| 3/8" | 0.0 | 0.0 | 100.0 |
| #4 | 0.0 | 0.0 | 100.0 |
| #10 | 1.6 | 1.5 | 98.5 |
| #40 | 11.3 | 10.7 | 89.3 |
| #200 | 8.7 | 29.7 | 70.3 |

| Elapsed Time (min) | Hydro Reading | Temp. Correct. Value | Corrected Hydro Reading | % Total in Susp. | K | Effective Length (cm) | Particle Diameter (mm) |
|---|---|---|---|---|---|---|---|
| 2 | 34.0 | 5.5 | 28.5 | 61.40 | 0.0132 | 11.64 | 0.0318 |
| 15 | 25.0 | 5.5 | 19.5 | 42.01 | 0.0132 | 13.11 | 0.0123 |
| 60 | 20.5 | 5.5 | 15 | 32.32 | 0.0132 | 13.85 | 0.0063 |
| 240 | 17.0 | 5.5 | 11.5 | 24.78 | 0.0132 | 14.42 | 0.0032 |
| 1440 | 13.0 | 5.5 | 7.5 | 16.16 | 0.0132 | 15.07 | 0.0014 |

**Summary of Grain Size Distribution**

| | |
|---|---|
| 0 | % GRAVEL |
| 2 | % COARSE SAND |
| 9 | % MEDIUM SAND |
| 19 | % FINE SAND |
| 41 | % SILT |
| 29 | % CLAY (<0.005mm) |

**Atterberg Limits**

| | |
|---|---|
| Liquid Limit | 34 |
| Plastic Limit | 22 |
| Plasticity Index | 12 |

**Natural Moisture Content**
18.3%

**Unified Soil Classification System**

| | |
|---|---|
| Group Name: | Lean Clay with Sand |
| Group Symbol: | CL |



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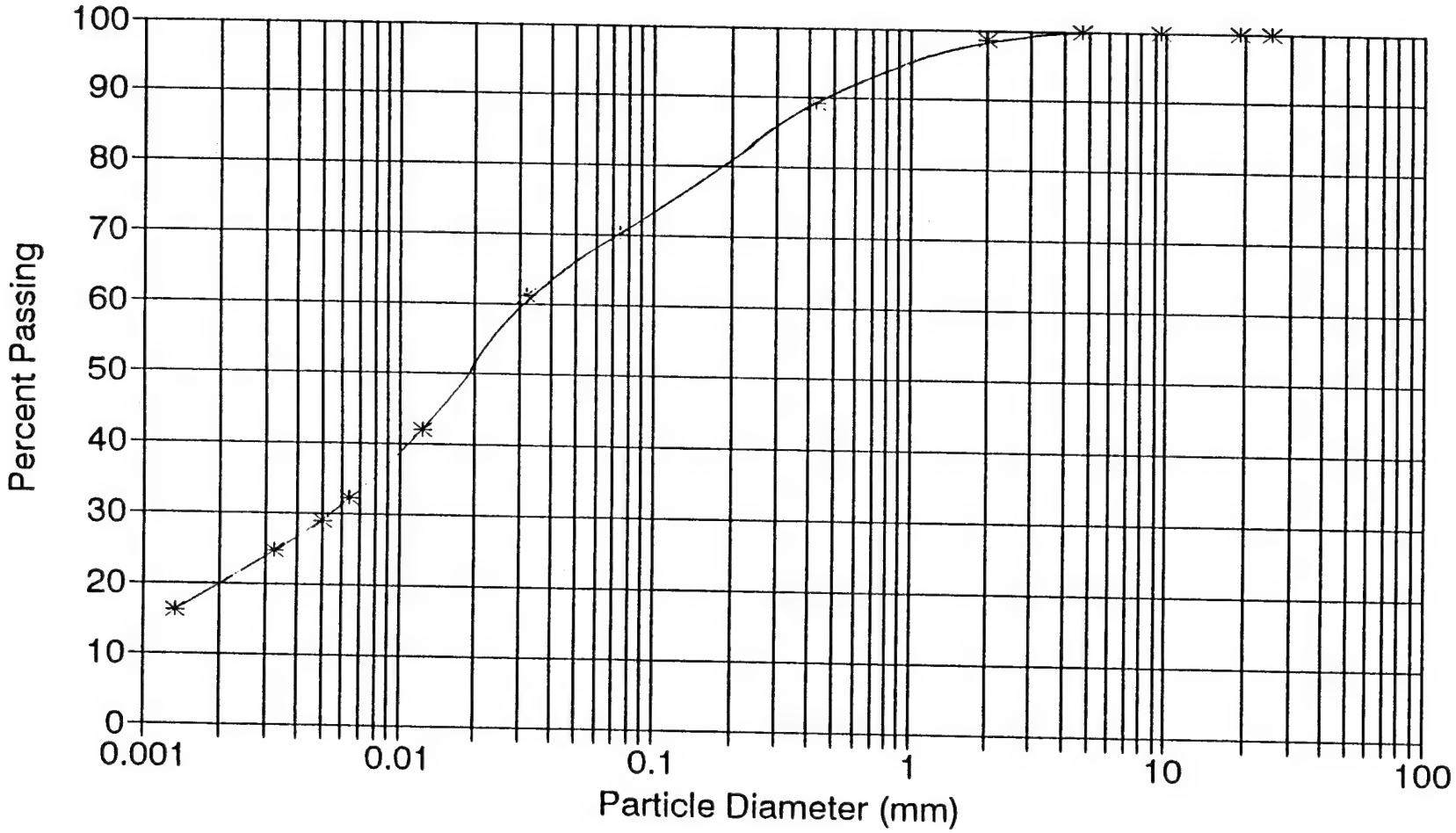
# Particle-Size Distribution
ASTM-D422

Jim Baker: Sample No. C



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Phone: 614/276-8123 · Fax: 614/276-6377



# Report on Sample of Soil

Project No.: 95050791 July 12, 1995

Client: Jim Baker
3275 Boxwood Drive
Fairborn, Ohio 45324

Lab ID No.: 95-30656

Identification: One soil sample submitted 6-20-95, identified as C.

---

TEST METHODS: pH by Method 9040 in USEPA Doc. SW 846, Total Organic Carbon by Combustion, Phosphate by Method 365.3, and Ammonia by Method 350.3, both from USEPA Doc. EPA-600/4-79-020, Nitrate by Ion Selective Electrode Potentiometry.

TEST RESULTS:

| Parameter | Result |
|---|---|
| pH | 7.82 S.U. |
| Total Organic Carbon | 5.91 % |
| Phosphate | 1.58 mg/kg |
| Ammonia as Nitrogen | 64.6 mg/kg |
| Nitrate as Nitrogen | 391 mg/kg |

QUALITY CONTROL DATA:

| Sample ID | Parameter | Reproducibility | Spike Recovery |
|---|---|---|---|
| 95-30656 A | pH | 100 % | -- |
| 95-30656 A | TOC | 103 % | -- |
| 95-30656 A | Phosphate | -- | 99 % |
| 95-30656 A | Ammonia | -- | 106 % |
| 95-30656 A | Nitrate | 98 % | -- |

---

Respectfully submitted,

Richard Herrold, Chemist

## Appendix C: Experiment Setup Documents

The following pages contain spreadsheet documents used to record certain activities preliminary to running the experiments. The two experiment runs were named "JIM 1" (17 June - 3 July 1995) and "JIM 2" (26 July - 9 August 1995). The first activity was the soil moisture tests, where the moisture content and field capacity of the three soils were determined. The other activy was the preparation of activated carbon tubes used to collect organic vapors from fuel that evaporates in the microcosms. The soil moisture data was used for both experiments. The organic vapors were measured exclusively by Thermo-gravimetric Analysis (TGA) in JIM 1 and by both TGA and direct gravimetric measurement in JIM 2.

# EXPERIMENT: JIM 1

***SOIL MOISTURE TESTS: 18 Jun 95***

| SOIL | Wt of Nat Soil | Tare | Tare + DrySoil | Wt of DrySoil | Residual Moisture | Soil as Used % Residual Moisture | Natural Soil % Moisture by Lab Test |
|---|---|---|---|---|---|---|---|
| A | 50.00 | 43.3154 | 83.21 | 39.8946 | 10.11 | 20.21 | 25.3 |
| B | 100.16 | 40.5919 | 125.216 | 84.6241 | 15.54 | 15.51 | 18.3 |
| C | 50.00 | 42.9408 | 86.06 | 43.1192 | 6.88 | 13.76 | 18.3 |

| JP8 | |
|---|---|
| spec gr$^{-1}$ | |
| ml/gr JP | 1.25 |

*FIELD CAPACITY TESTS: 18-19 Jun 95*

| SOIL | Tare Column | Wt Col + DrySoil | Wt of DrySoil | Col + WetSoil | Wt of wet soil@ 100% f cap | Wt of water @ 100% f cap | % Moisture @ 100% f cap | (per 100g soil) wt of water to make 60% f cap | (per final sample) ml JP-8 to make treatment by weight 1% | 0.10% |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 112.6897 | 152.5612 | 39.8715 | 174.4425 | 61.7528 | 21.8813 | 35.4337 | 1.3328 | 1.2667 | 0.1267 |
| B | 112.7318 | 156.6085 | 43.8767 | 172.2400 | 59.5082 | 15.6315 | 26.2678 | 0.2963 | 1.2537 | 0.1254 |
| C | 113.3945 | 155.1777 | 41.7832 | 178.4485 | 65.0540 | 23.2708 | 35.7715 | 9.8059 | 1.3726 | 0.1373 |

***SOIL CHARACTERISTICS as determined by commercial lab (samples submitted 20 Jun 95)***

| SOIL | Summary of Grain Size Distribution (%) Gravel | Crs Sand | Med Sand | Fine Sand | Silt | Clay | Classification | pH | TOC (%) | mg/kg $PO_4$ | $NH_3$ | $NO_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 3 | 5 | 13 | 24 | 39 | 16 | *Sandy Silt (ML)* | 7.92 | 7.04 | 2.80 | 20.3 | 280 |
| B | 0 | 0 | 40 | 45 | 9 | 6 | *Sandy Silt (ML)* | 8.08 | 2.05 | 2.60 | 11.7 | 336 |
| C | 0 | 2 | 9 | 19 | 41 | 29 | *Lean Clay w/ Sand (CL)* | 7.82 | 5.91 | 1.58 | 64.6 | 391 |

# EXPERIMENT: JIM 1

*All weights in grams unless otherwise noted.*

## PREPARATION OF ACTIVATED CARBON ORGANIC VAPOR RECOVERY TUBES: 17 Jun 95

| Tube No. | Tare | Tare plus Carbon | Original Wt of Carbon | TGA Avg Wt Loss Rate Org Vap* | Total Org Vapors Trapped | Trtmnt: Soil; % Fuel | Micro-cosm | Fuel Originally Added | Fraction recovered as vapor |
|---|---|---|---|---|---|---|---|---|---|
| A | 14.9203 | 17.2714 | 2.3511 | 0.0332 | 0.0807 | A; 1 | 16 | 1.0134 | 0.080 |
| B | 13.1548 | 15.5890 | 2.4342 | 0.0000 | 0.0000 | B; 0.1 | 11 | 0.1003 | 0.000 |
| C | 12.1010 | 14.3602 | 2.2592 | 0.0827 | 0.2037 | C; 1 | 20 | 1.0981 | 0.185 |
| D | 15.5708 | 17.8729 | 2.3021 | 0.0000 | 0.0000 | B; 0.1 | 1 | 0.1003 | 0.000 |
| E | 15.2587 | 17.7043 | 2.4456 | 0.0814 | 0.2167 | A; 1 | 9 | 1.0134 | 0.214 |
| F | 16.0911 | 18.5730 | 2.4819 | 0.0787 | 0.2120 | C; 1 | 19 | 1.0981 | 0.193 |
| G | 15.2786 | 17.7625 | 2.4839 | 0.0000 | 0.0000 | control | na | na | na |
| H | 13.2376 | 16.1114 | 2.8738 | 0.0182 | 0.0533 | A; 0.1 | 3 | 0.1013 | 0.526 |
| I | 15.6034 | 18.2309 | 2.6275 | 0.0070 | 0.0185 | C; 0.1 | 13 | 0.1098 | 0.169 |
| J | 15.6634 | 18.0824 | 2.4190 | 0.0792 | 0.2081 | B; 1 | 18 | 1.003 | 0.207 |
| K | 12.1782 | 14.5323 | 2.3541 | 0.0081 | 0.0192 | C; 0.1 | 6 | 0.1003 | 0.192 |
| L | 15.5722 | 17.9519 | 2.3797 | 0.0885 | 0.2311 | B; 1 | 14 | 1.003 | 0.230 |
| M | 14.8614 | 17.3111 | 2.4497 | 0.0259 | 0.0651 | A; 0.1 | 10 | 0.1013 | 0.643 |
| N | 15.2199 | 17.6767 | 2.4568 | 0.0000 | 0.0000 | B; 0 | 12 | 0 | 0.000 |
| O | 12.2113 | 14.6452 | 2.4339 | 0.0000 | 0.0000 | C; 0 | 7 | 0 | 0.000 |
| P | 15.8918 | 18.4445 | 2.5527 | 0.0000 | 0.0000 | control | na | na | na |
| Q | 15.5448 | 17.8974 | 2.3526 | 0.0000 | 0.0000 | C; 0 | 2 | 0 | 0.000 |
| R | 15.4678 | 17.7082 | 2.2404 | 0.0000 | 0.0000 | A; 0 | 5 | 0 | 0.000 |
| S | 12.6366 | 15.1587 | 2.5221 | 0.0000 | 0.0000 | A; 0 | 8 | 0 | 0.000 |
| T | 12.5299 | 14.8896 | 2.3597 | 0.0000 | 0.0000 | B; 0 | 15 | 0 | 0.000 |

No organic vapor tubes on microcosms 4 & 17 (empty bottles).
Tubes G & P were not placed in line, and served as controls.

## EXPERIMENT SETUP: JIM 2

*All weights in grams unless otherwise noted.*

## PREPARATION OF ACTIVATED CARBON ORGANIC VAPOR RECOVERY TUBES: 26 Jul 95

| Tube No. | Tare | Tare plus Carbon | Original Weight Carbon | Micro-cosm* | Treat-ment | Final Weight Carbon | Correct Lost Carbon** | Corr. Final Weight Carbon | Wt Gain | Gain Correct Moisture 3% | Adj Gain from controls | TGA Wt Loss Rate due to Org Vap | Total Org Vap Trapped gm |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA | 12.9708 | 15.5195 | 2.5487 | 7 | A; 1 | **TGA** | 0.0000 | ~ | ~ | ~ | ~ | 0.17616 | 0.5450 |
| BB | 13.2228 | 15.8277 | 2.6049 | 18 | C; 0 | 2.5959 | 0.0016 | 2.5975 | -0.0074 | 2.5196 | 0.0574 | ~ | ~ |
| CC | 13.4468 | 15.9363 | 2.4895 | 20 | C; 0 | 2.4900 | 0.0016 | 2.4916 | 0.0021 | 2.4168 | 0.0666 | ~ | ~ |
| DD | 12.7428 | 15.1601 | 2.4173 | 10 | B; 0 | 2.4196 | 0.0016 | 2.4212 | 0.0039 | 2.3485 | 0.0684 | ~ | ~ |
| EE | 13.0811 | 15.7324 | 2.6513 | 3 | B; 0.1 | 2.6735 | 0.0016 | 2.6751 | 0.0238 | 2.5948 | 0.0877 | ~ | 0.0877 |
| FF | 13.0570 | 15.8310 | 2.7740 | 4 | B; 0.1 | 2.7836 | 0.0016 | 2.7852 | 0.0112 | 2.7016 | 0.0754 | ~ | 0.0754 |
| GG | 12.6710 | 14.9287 | 2.2577 | **ctrl** | na | 2.2913 | 0.0000 | 2.2913 | 0.0336 | 2.2226 | ~ | ~ | na |
| HH | 12.9056 | 15.3543 | 2.4487 | 13 | C; 1 | **TGA** | 0.0016 | ~ | ~ | ~ | ~ | 0.13329 | 0.3766 |
| II | 13.5129 | 16.1283 | 2.6154 | 12 | A; 0 | 2.6065 | 0.0016 | 2.6081 | -0.0073 | 2.5298 | 0.0575 | ~ | ~ |
| JJ | 12.4220 | 14.6850 | 2.2630 | 6 | A; 0.1 | **TGA** | 0.0016 | ~ | ~ | ~ | ~ | 0.01974 | 0.0456 |
| KK | 12.6044 | 14.9605 | 2.3561 | 9 | B; 0 | 2.3510 | 0.0016 | 2.3526 | -0.0035 | 2.2820 | 0.0612 | ~ | ~ |
| LL | 15.4852 | 17.7271 | 2.2419 | 15 | A; 0 | 2.2369 | 0.0016 | 2.2385 | -0.0034 | 2.1713 | 0.0613 | ~ | ~ |
| MM | 12.8583 | 15.3646 | 2.5063 | 5 | C; 0.1 | 2.5059 | 0.0016 | 2.5075 | 0.0012 | 2.4323 | 0.0657 | ~ | 0.0657 |
| NN | 13.2513 | 15.9641 | 2.7128 | 8 | B; 1 | 2.8914 | 0.0016 | 2.8930 | 0.1802 | 2.8062 | 0.2394 | ~ | 0.2394 |
| OO | 13.5599 | 16.4236 | 2.8637 | 19 | C; 1 | 3.0568 | 0.0016 | 3.0584 | 0.1947 | 2.9666 | 0.2534 | ~ | 0.2534 |
| PP | 13.0850 | 15.6720 | 2.5870 | **ctrl** | na | 2.6180 | 0.0000 | 2.6180 | 0.0310 | 2.5395 | ~ | ~ | na |
| QQ | 12.7032 | 15.0116 | 2.3084 | 11 | A; 0.1 | 2.3358 | 0.0016 | 2.3374 | 0.0290 | 2.2673 | 0.0927 | ~ | 0.0927 |
| RR | 13.0739 | 15.6061 | 2.5322 | 1 | C; 0.1 | 2.5273 | 0.0016 | 2.5289 | -0.0033 | 2.4530 | 0.0614 | ~ | 0.0614 |
| SS | 12.7676 | 15.1150 | 2.3474 | 14 | B; 1 | 2.5283 | 0.0016 | 2.5299 | 0.1825 | 2.4540 | 0.2416 | ~ | 0.2416 |
| TT | 15.7075 | 18.0931 | 2.3856 | 17 | A; 1 | 2.5489 | 0.0016 | 2.5505 | 0.1649 | 2.4740 | 0.2245 | ~ | 0.2245 |

* - microcosms 2, 16 are empty bottles

**- avg. wt. of carbon dust retained on 2 filters and in tube (.0016 gm) determined by gravimetric analysis.

*** the two controls (GG, PP) gained an avg of 0.0323 gm

# ASSIGNMENT OF TREATMENTS TO MICROCOSMS: JIM 1 & 2

**ASSIGNMENT MATRIX**

| SOIL TYPE | JP8 Conc 1% | 0.10% | 0% |
|---|---|---|---|
| A | 1 | 2 | 3 |
| B | 4 | 5 | 6 |
| C | 7 | 8 | 9 |
| | 0* | | |

**RANDOMLY-ASSIGNED TREATMENTS**

| | ~ ~ ~ JIM 1 ~ ~ ~ | | | ~ ~ ~ JIM 2 ~ ~ ~ | | |
|---|---|---|---|---|---|---|
| SOIL TYPE | JP8 Conc 1 percent | 0.1 percent | 0 percent | JP8 Conc 1 percent | 0.1 percent | 0 percent |
| A | 9, 16 | 3, 10 | 5, 8 | 7, 17 | 6, 11 | 12, 15 |
| B | 14, 18 | 1, 11 | 12, 15 | 8, 14 | 3, 4 | 9, 10 |
| C | 19, 20 | 6, 13 | 2, 7 | 13, 19 | 1, 5 | 18, 20 |
| | 4*, 17* | | | 2*, 16* | | |

* - empty microcosms

## Appendix D: ANOVA Tests--Fuel & Clay vs. $O_2$ Uptake

The following are the Statistix™ statistics package outputs and the construction of the tests of hypotheses for each element of the analysis. Also included are the comparison of means tests performed on the responses to the complete range of each factor's levels.

The cumulative oxygen data for each treatment was arranged by fuel level, then by clay level, then by replication number. The Statistix™ package then partitioned the sums of squares and then computed the mean squares, degrees of freedom, F statistics, p values, and other data needed to test the various hypotheses. The raw data follows the outputs.

ANALYSIS OF VARIANCE TABLE FOR CUMO2

| SOURCE | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| FUEL (A) | 2 | 5.294E+10 | 2.647E+10 | 312.89 | 0.0000 |
| CLAY (B) | 2 | 1.727E+10 | 8.636E+09 | 102.09 | 0.0000 |
| A*B | 4 | 5.833E+09 | 1.458E+09 | 17.24 | 0.0000 |
| RESIDUAL | 27 | 2.284E+09 | 8.460E+07 | | |
| TOTAL | 35 | 7.833E+10 | | | |

TEST ($\alpha = .05$)

$H_0$: FUEL and CLAY do not interact to affect CUMO2
$H_a$: FUEL and CLAY do interact

Rejection region, $F > F_{\alpha, v1, v2}$

Mean Square for Interaction, MS(AB) = 1.458E+09
Mean Square for Error, MSE = 8.460E+07

F Statistic for Interaction = MS(AB)/MSE = 17.234

$F_{\alpha, v1, v2} = F_{0.05, 4, 27} = 2.73$

**17.234 > 2.73 therefore reject $H_0$ factors do interact**

TABLE D.1

RAW DATA: MEANS (uL) OF TOTAL CO2 AND O2 FROM EACH TREATMENT

FUEL: 3 = 1%, 2 = 0.1%, 1 = 0%
SOIL (SOIL #, % CLAY): 3 = C, 29, 2 = A, 16, 1 = B, 6
REPLICATION: 1, 2, 3, 4

| CASE | CUMCO2 | CUMO2 | FUEL | REPL | SOIL |
|---|---|---|---|---|---|
| 1 | 57316.0 | 174084.0 | 3.0000 | 1.0000 | 3.0000 |
| 2 | 59390.0 | 183904.0 | 3.0000 | 2.0000 | 3.0000 |
| 3 | 54712.0 | 193644.0 | 3.0000 | 3.0000 | 3.0000 |
| 4 | 54854.0 | 189249.0 | 3.0000 | 4.0000 | 3.0000 |
| 5 | 33733.0 | 100607.0 | 2.0000 | 1.0000 | 3.0000 |
| 6 | 29761.0 | 92756.0 | 2.0000 | 2.0000 | 3.0000 |
| 7 | 35280.0 | 98295.0 | 2.0000 | 3.0000 | 3.0000 |
| 8 | 35328.0 | 102043.0 | 2.0000 | 4.0000 | 3.0000 |
| 9 | 21295.0 | 58464.0 | 1.0000 | 1.0000 | 3.0000 |
| 10 | 19906.0 | 55181.0 | 1.0000 | 2.0000 | 3.0000 |
| 11 | 21662.0 | 60682.0 | 1.0000 | 3.0000 | 3.0000 |
| 12 | 29155.0 | 82206.0 | 1.0000 | 4.0000 | 3.0000 |
| 13 | 49042.0 | 141620.0 | 3.0000 | 1.0000 | 2.0000 |
| 14 | 49600.0 | 146503.0 | 3.0000 | 2.0000 | 2.0000 |
| 15 | 45729.0 | 149147.0 | 3.0000 | 3.0000 | 2.0000 |
| 16 | 47275.0 | 143714.0 | 3.0000 | 4.0000 | 2.0000 |
| 17 | 22695.0 | 71240.0 | 2.0000 | 1.0000 | 2.0000 |
| 18 | 22185.0 | 74963.0 | 2.0000 | 2.0000 | 2.0000 |
| 19 | 26396.0 | 74412.0 | 2.0000 | 3.0000 | 2.0000 |
| 20 | 27529.0 | 75443.0 | 2.0000 | 4.0000 | 2.0000 |
| 21 | 13318.0 | 38797.0 | 1.0000 | 1.0000 | 2.0000 |
| 22 | 13067.0 | 34650.0 | 1.0000 | 2.0000 | 2.0000 |
| 23 | 16617.0 | 47670.0 | 1.0000 | 3.0000 | 2.0000 |
| 24 | 17882.0 | 50406.0 | 1.0000 | 4.0000 | 2.0000 |
| 25 | 29524.0 | 86142.0 | 3.0000 | 1.0000 | 1.0000 |
| 26 | 28298.0 | 82030.0 | 3.0000 | 2.0000 | 1.0000 |
| 27 | 34197.0 | 96314.0 | 3.0000 | 3.0000 | 1.0000 |
| 28 | 35360.0 | 92932.0 | 3.0000 | 4.0000 | 1.0000 |
| 29 | 17382.0 | 58114.0 | 2.0000 | 1.0000 | 1.0000 |
| 30 | 16930.0 | 54275.0 | 2.0000 | 2.0000 | 1.0000 |
| 31 | 23310.0 | 67771.0 | 2.0000 | 3.0000 | 1.0000 |
| 32 | 21488.0 | 63820.0 | 2.0000 | 4.0000 | 1.0000 |
| 33 | 9823.0 | 28621.0 | 1.0000 | 1.0000 | 1.0000 |
| 34 | 10246.0 | 27235.0 | 1.0000 | 2.0000 | 1.0000 |
| 35 | 22449.0 | 65608.0 | 1.0000 | 3.0000 | 1.0000 |
| 36 | 9498.0 | 24857.0 | 1.0000 | 4.0000 | 1.0000 |

**TUKEY PAIRWISE COMPARISON OF MEANS**

**Fuel & Soil Factors on O2 Uptake Response**

| | |
|---|---|
| Level of significance, | $\alpha = 0.05$ |
| Levels of factor a | $a = 3$ |
| Levels of factor b | $b = 3$ |
| Number of replications | $n = 4$ |
| MSE from 2-way ANOVA | MSE = 8.46E+07 |
| Variance of $D_{hat}$ (2MSE/n) | $s^2\{D_{hat}\}$ = 4.23E+07 |
| Std Deviation of $D_{hat}$ | $s\{D_{hat}\}$ = 6504 |

The difference between means, $D = \mu_{ij} - \mu_{i'j'}$

The Tukey multiple, T = 3.3694

$$T = \frac{1}{\sqrt{2}} q[1-a;\ ab,\ (n-1)ab]$$

The student's t: q(.95; 9, 27) = 4.765

The confidence interval 95%CI = ± $T*s\{D_{hat}\}$

**95%CI = ± 21914**

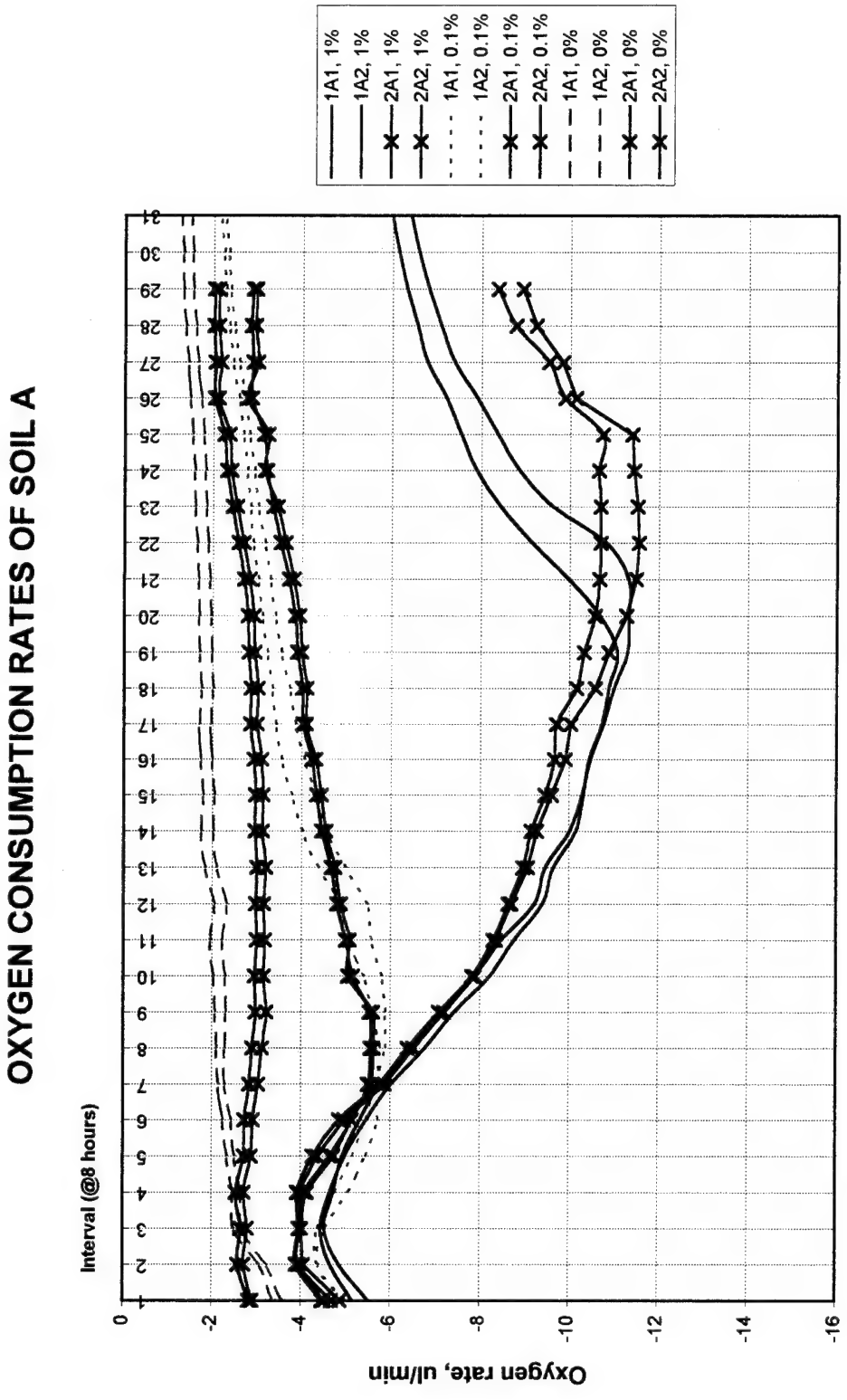
| THE DATA | | | |
|---|---|---|---|
| **FACTORS** | LEVELS | | |
| Soil / Fuel | 0 | 0.1 | 1 |
| C | 64133 | 98425 | 185220 |
| B | 42881 | 74015 | 145246 |
| A | 36580 | 60995 | 89354 |

If D, the difference beween each pair, is greater than half the confidence interval, then there is a significant difference between the pairs.

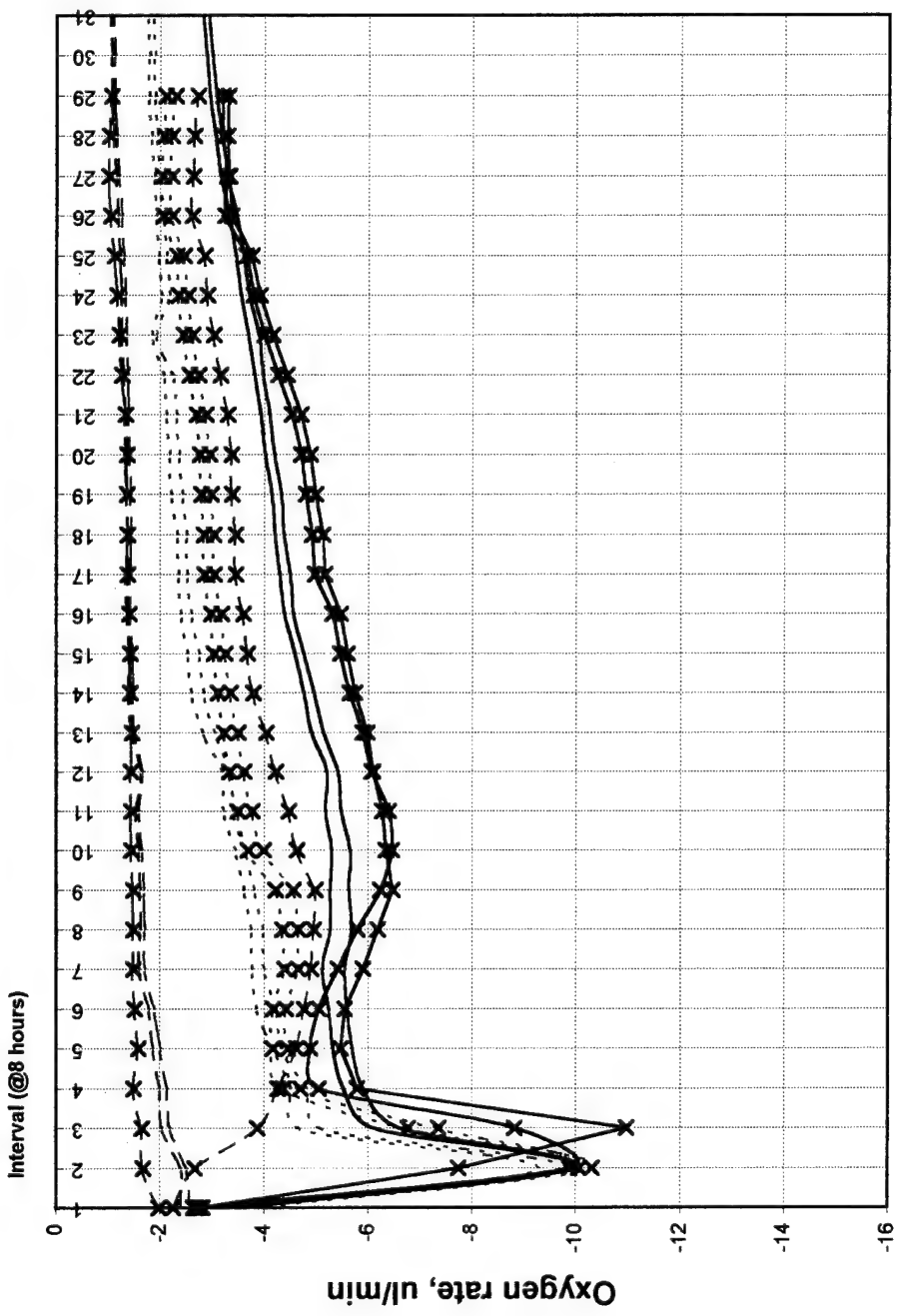
| PAIR | DIFF. | Half CI | SIG DIFF? |
|---|---|---|---|
| 0, CB | 21252 | 21914 | N |
| 0, CA | 27553 | 21914 | Y |
| 0, AB | 6301 | 21914 | N |
| 0.1, CB | 24410 | 21914 | Y |
| 0.1, CA | 37430 | 21914 | Y |
| 0.1, AB | 13020 | 21914 | N |
| 1, CB | 39974 | 21914 | Y |
| 1, CA | 95866 | 21914 | Y |
| 1, AB | 55892 | 21914 | Y |

## Appendix E: Respirometer Curves

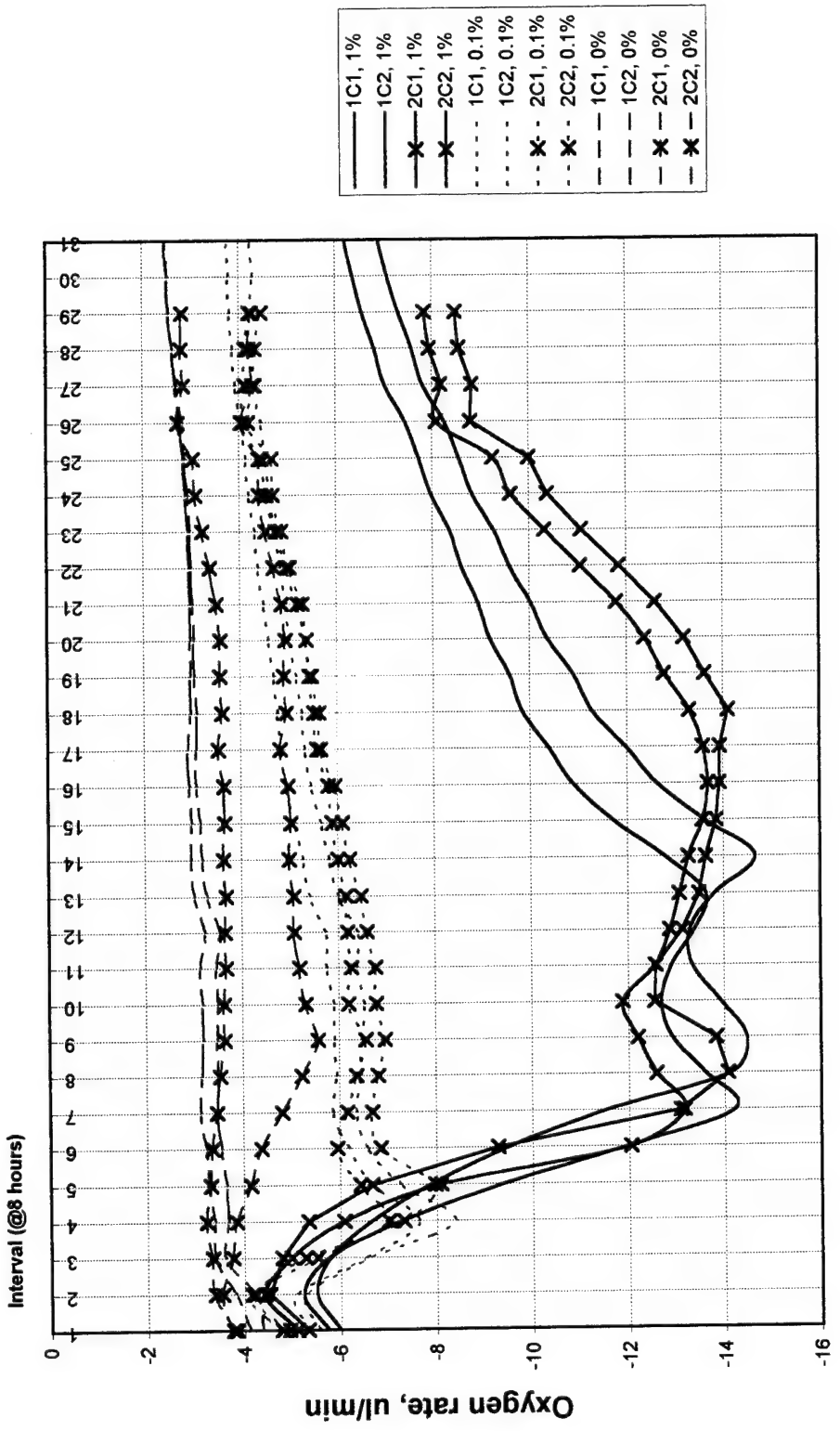
The following pages contain the respiration rate and cumulative consumption (and production) curves for all the treatments from the experiments. The oxygen curves are shown, since these were the ones on which the biodegradation was based. (These raw data graphs show oxygen consumption as negative numbers, as the data came from the respirometer.) The carbon dioxide curves were generally mirror images of the oxygen curves and about one-third the scale. The single line curves were prepared from the means of the four replications of the treatments, except for the "Four-hour Interval" curves, which are from two replications from the first two days of run "JIM 2" only. The vertical scale of each curve was kept the same within each family of curves to facilitate comparisons. The following is a table of contents for the families of curves that follow:

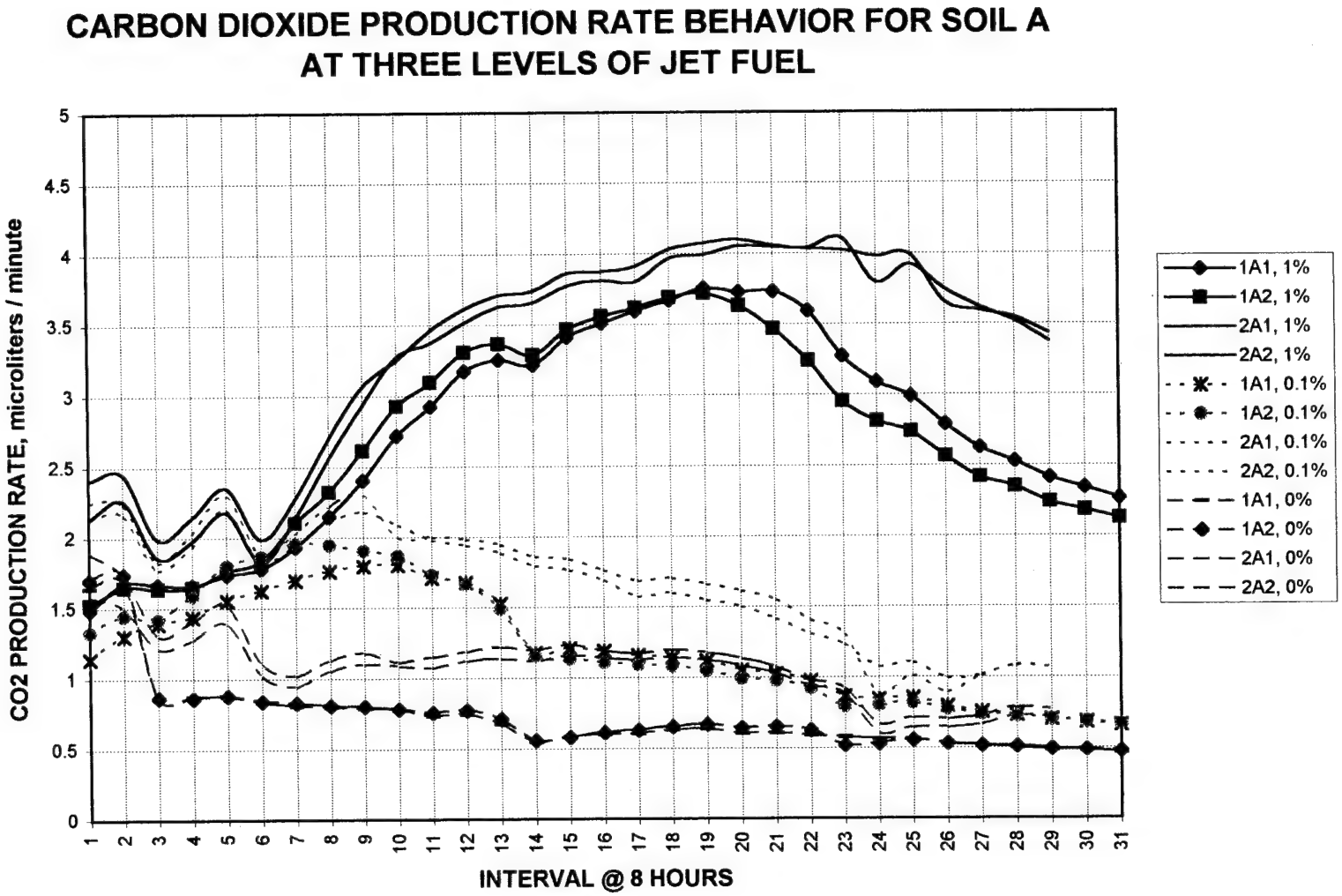

OXYGEN CONSUMPTION RATES OF SOIL A
Interval (@8 hours)
Oxygen rate, ul/min
1A1, 1%
1A2, 1%
2A1, 1%
2A2, 1%
1A1, 0.1%
1A2, 0.1%
2A1, 0.1%
2A2, 0.1%
1A1, 0%
1A2, 0%
2A1, 0%
2A2, 0%

# OXYGEN CONSUMPTION RATES OF SOIL B

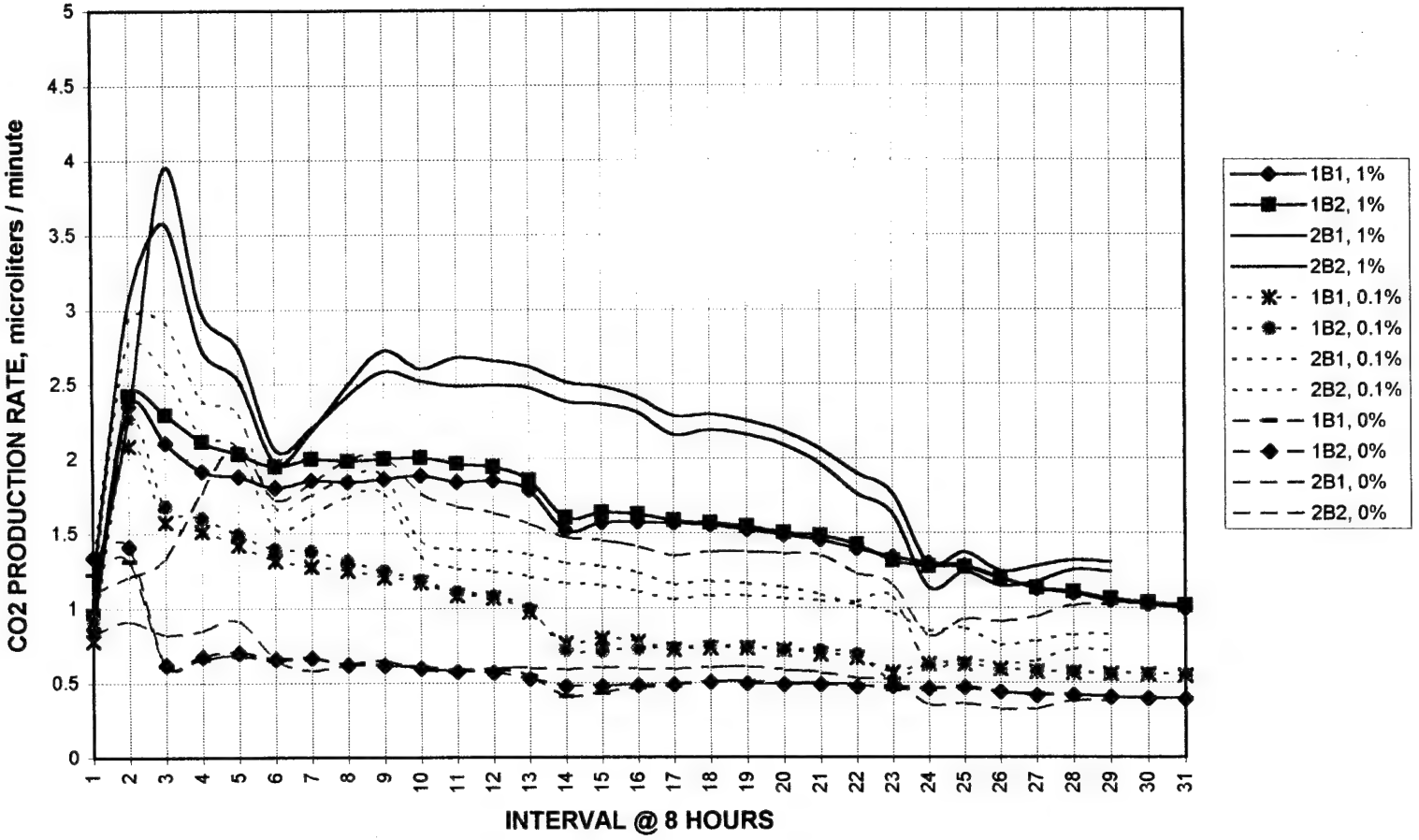


# OXYGEN CONSUMPTION RATES OF SOIL C

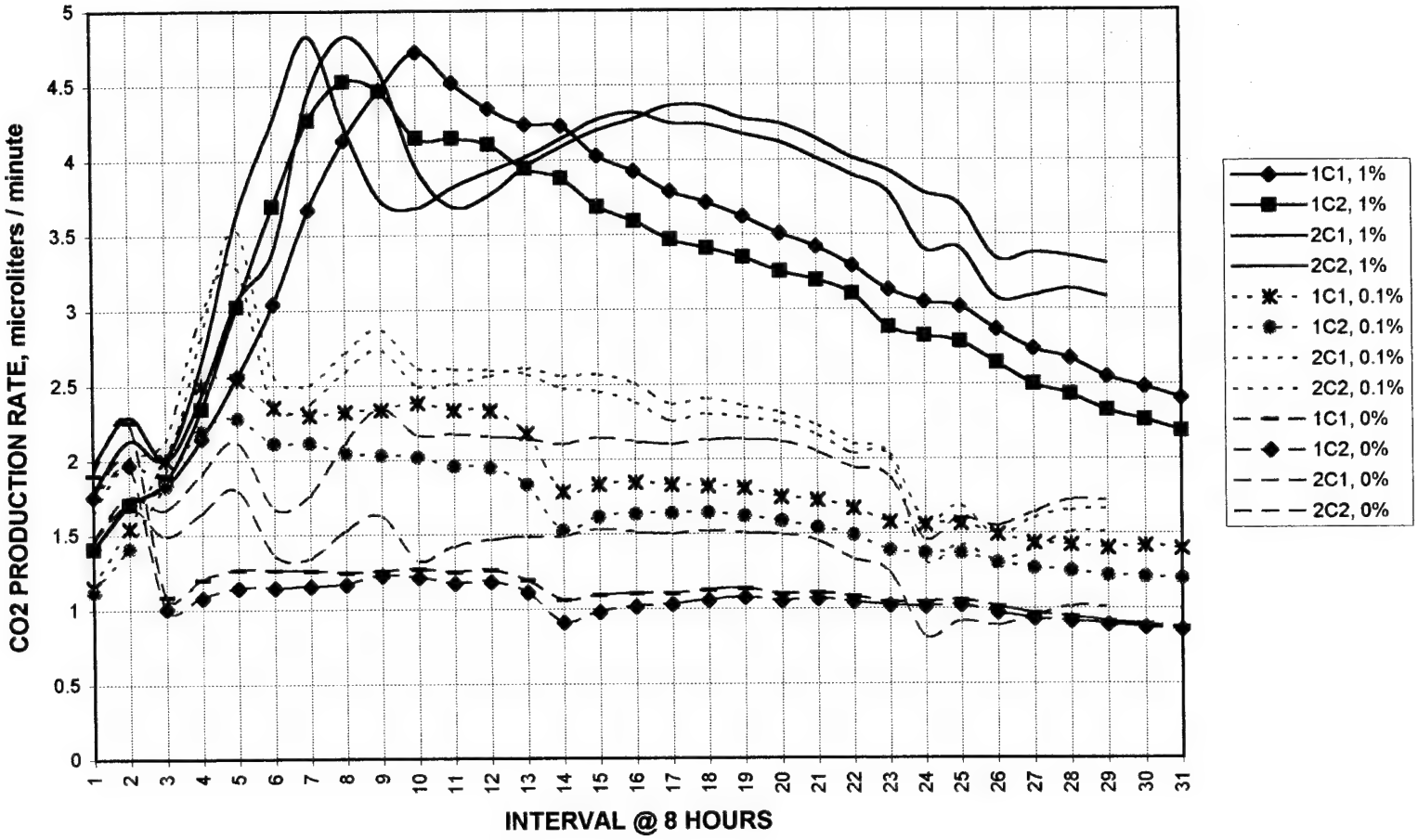


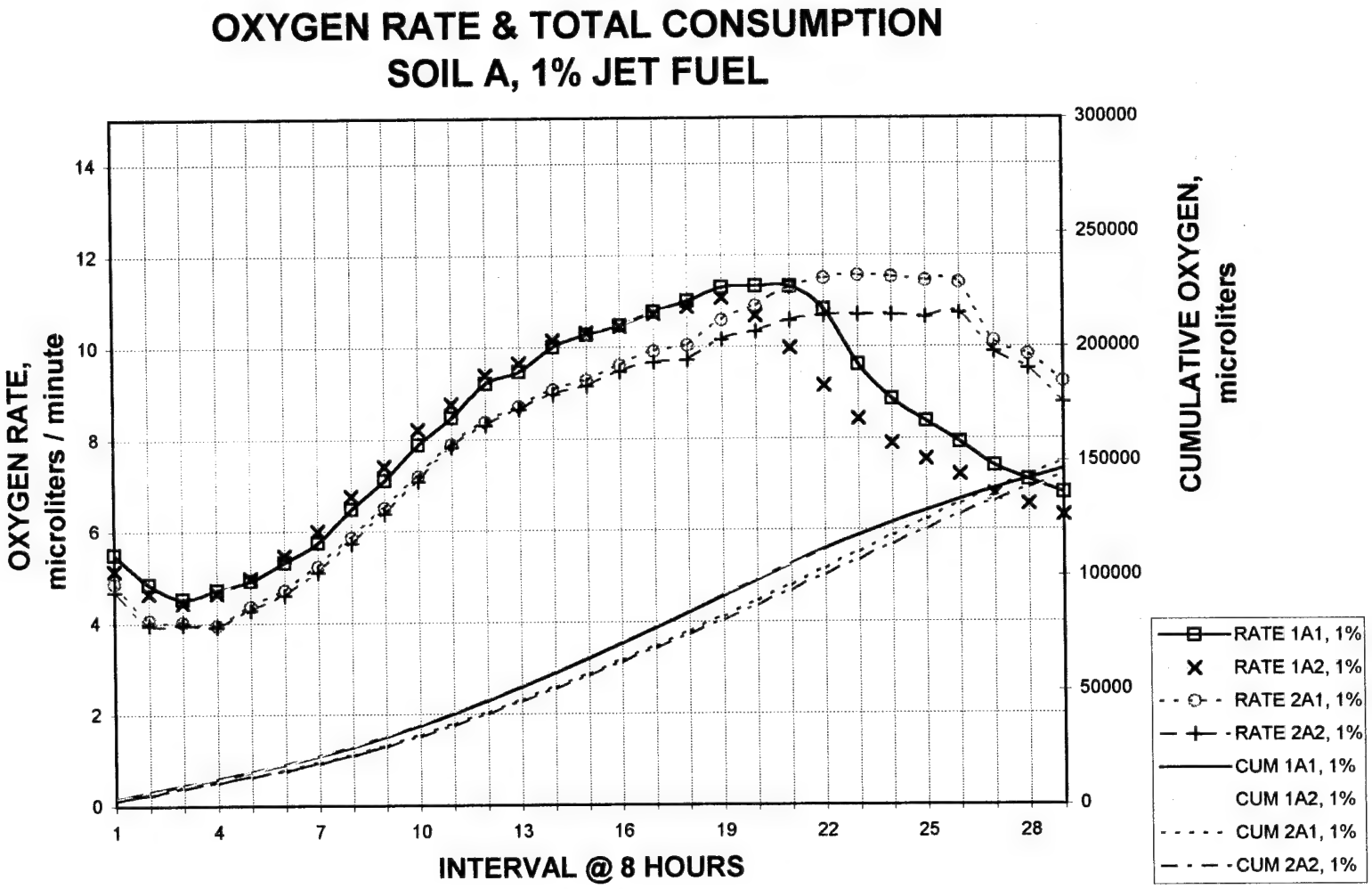

CARBON DIOXIDE PRODUCTION RATE BEHAVIOR FOR SOIL A
AT THREE LEVELS OF JET FUEL
CO2 PRODUCTION RATE, microliters / minute
INTERVAL @ 8 HOURS
1A1, 1%
1A2, 1%
2A1, 1%
2A2, 1%
1A1, 0.1%
1A2, 0.1%
2A1, 0.1%
2A2, 0.1%
1A1, 0%
1A2, 0%
2A1, 0%
2A2, 0%

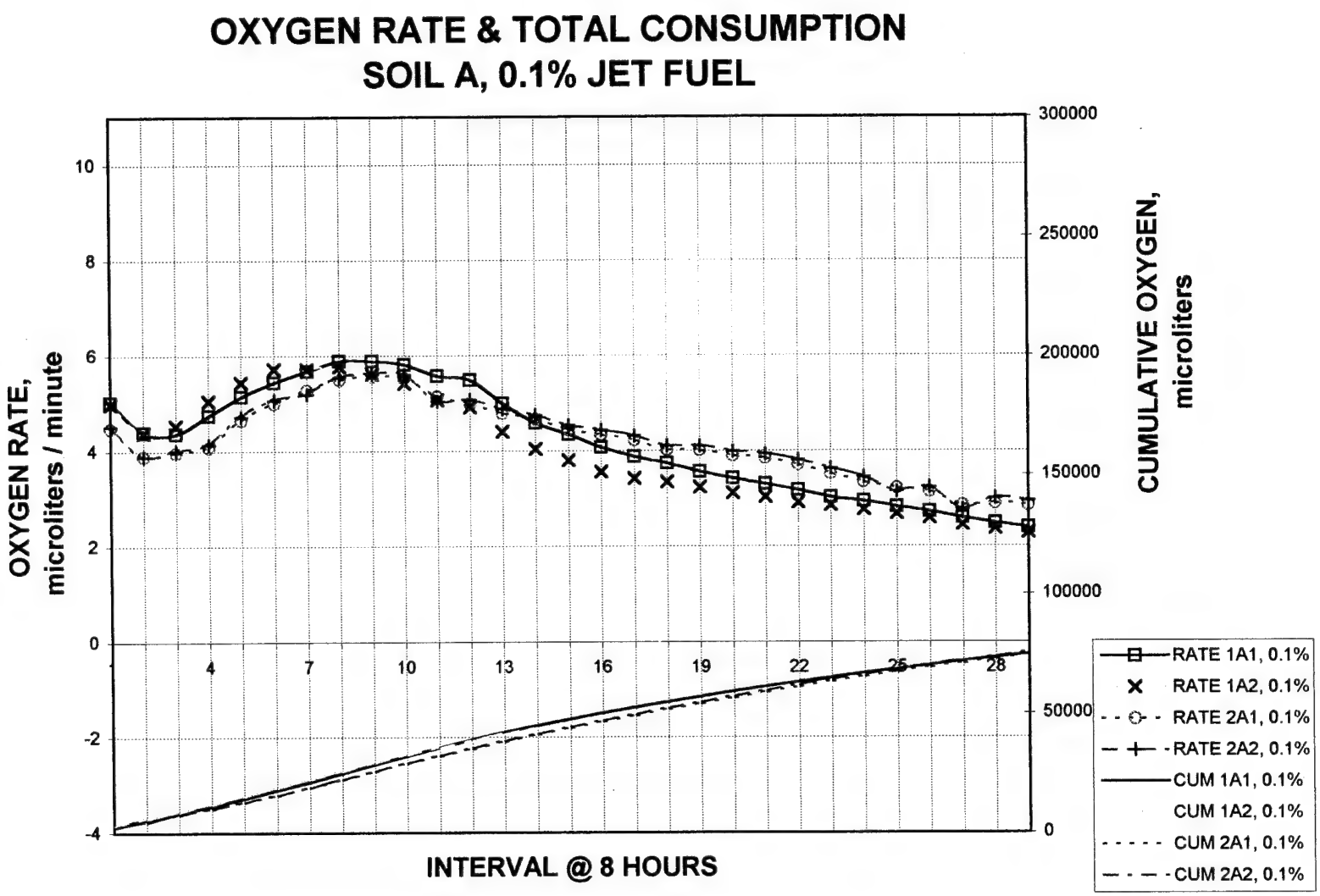
## CARBON DIOXIDE PRODUCTION RATE BEHAVIOR FOR SOIL B AT THREE LEVELS OF JET FUEL

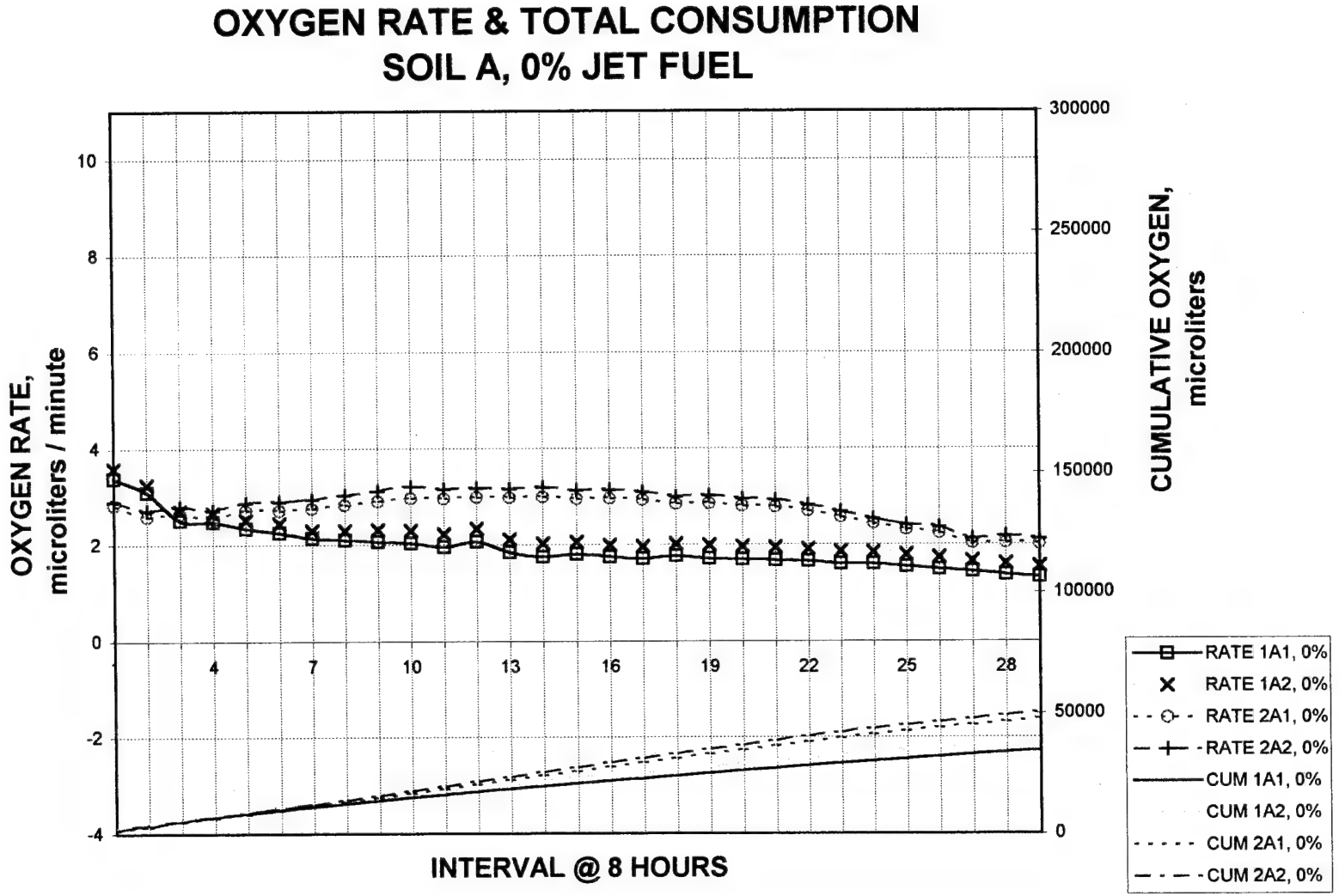


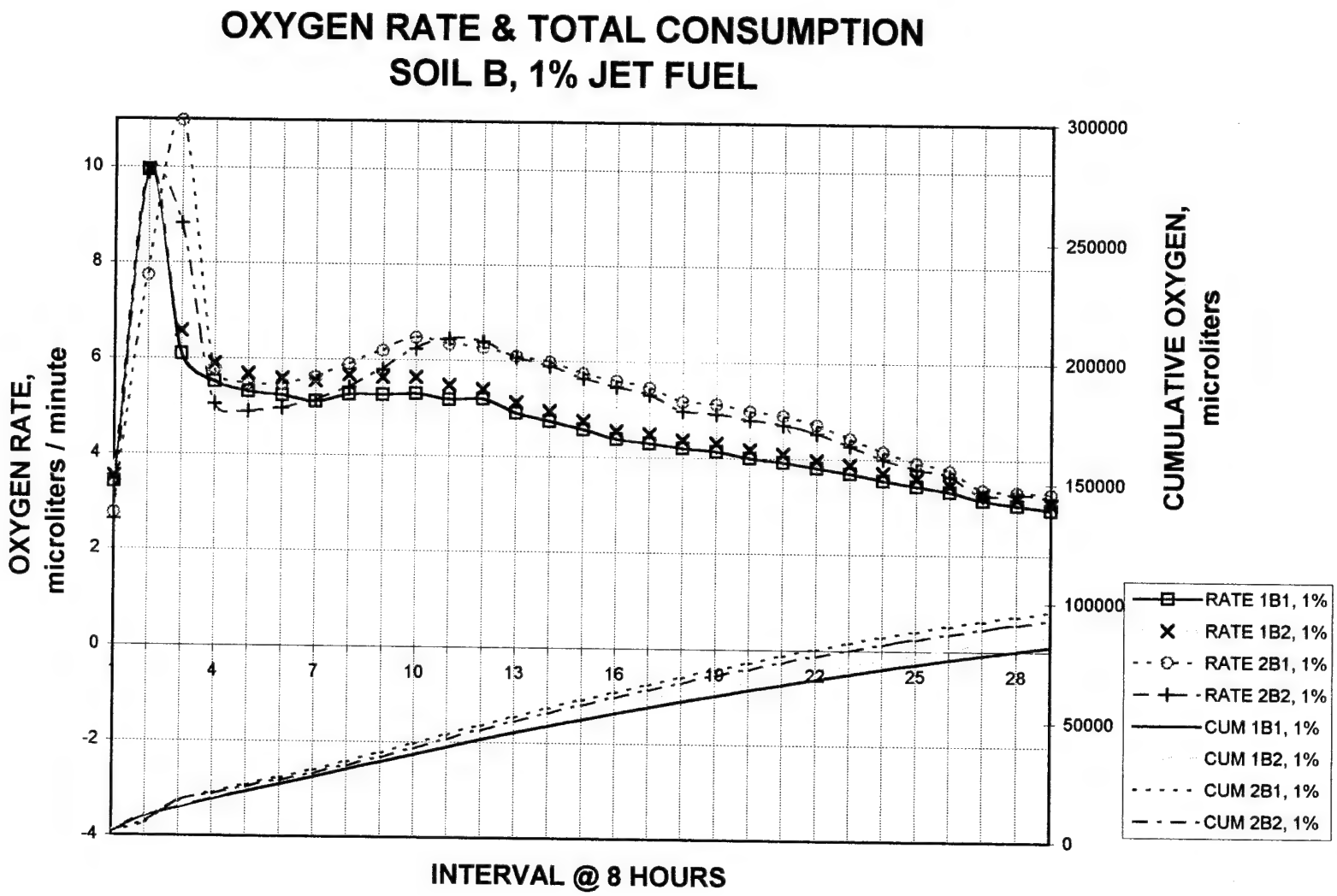
# CARBON DIOXIDE PRODUCTION RATE BEHAVIOR FOR SOIL C AT THREE LEVELS OF JET FUEL

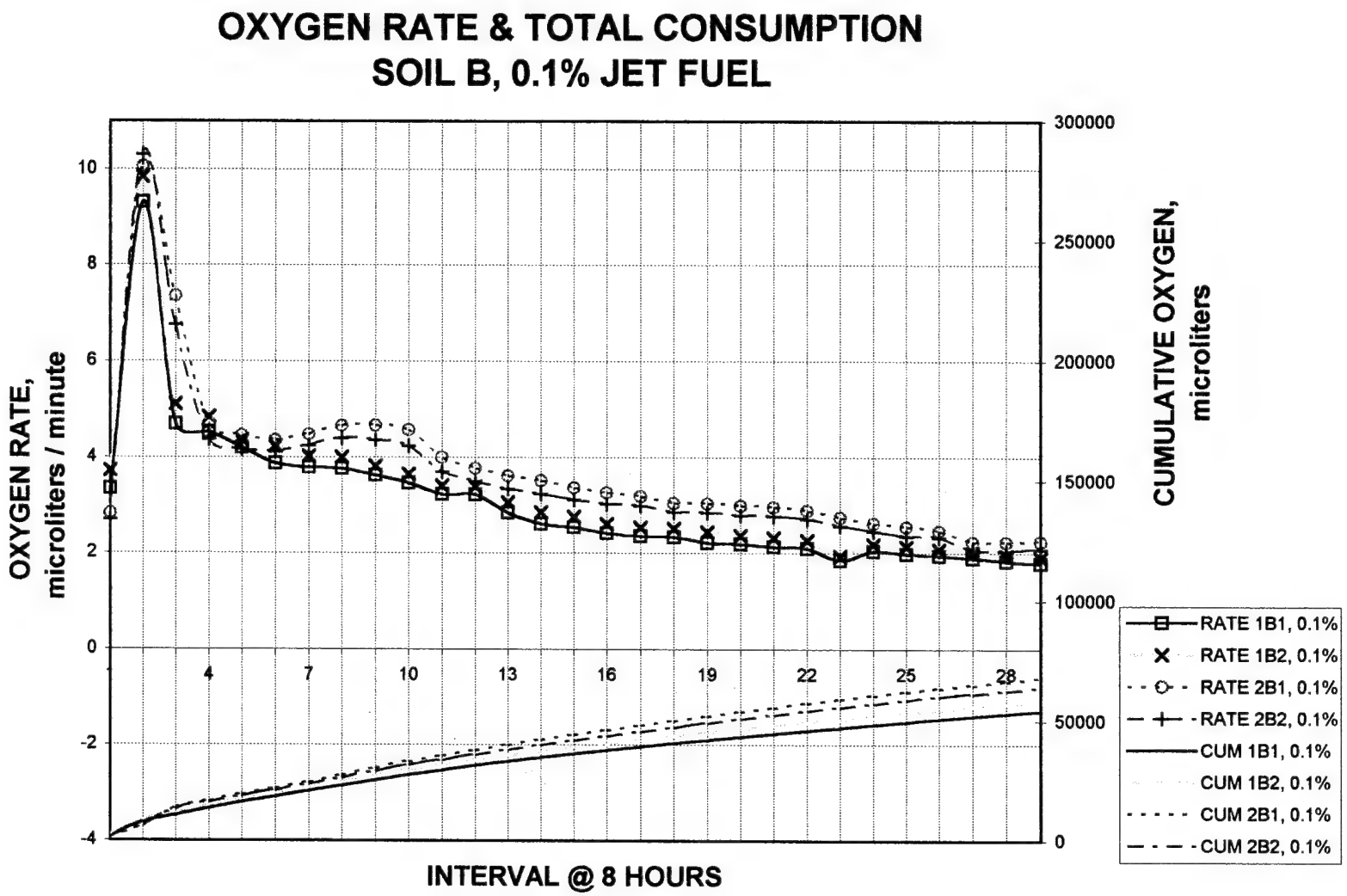


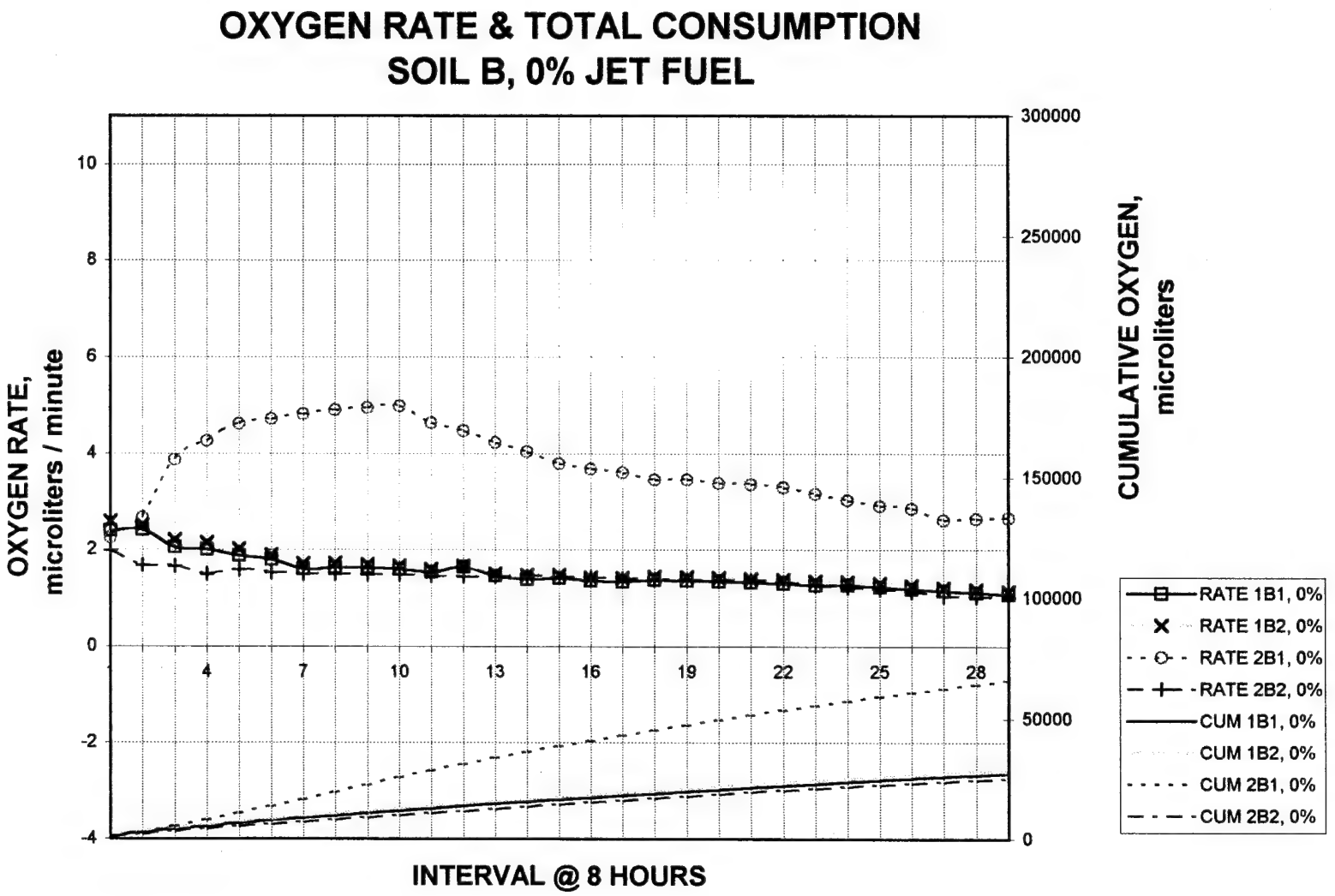

OXYGEN RATE & TOTAL CONSUMPTION
SOIL A, 1% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
0
2
4
6
8
10
12
14
0
50000
100000
150000
200000
250000
300000
1
4
7
10
13
16
19
22
25
28
RATE 1A1, 1%
RATE 1A2, 1%
RATE 2A1, 1%
RATE 2A2, 1%
CUM 1A1, 1%
CUM 1A2, 1%
CUM 2A1, 1%
CUM 2A2, 1%

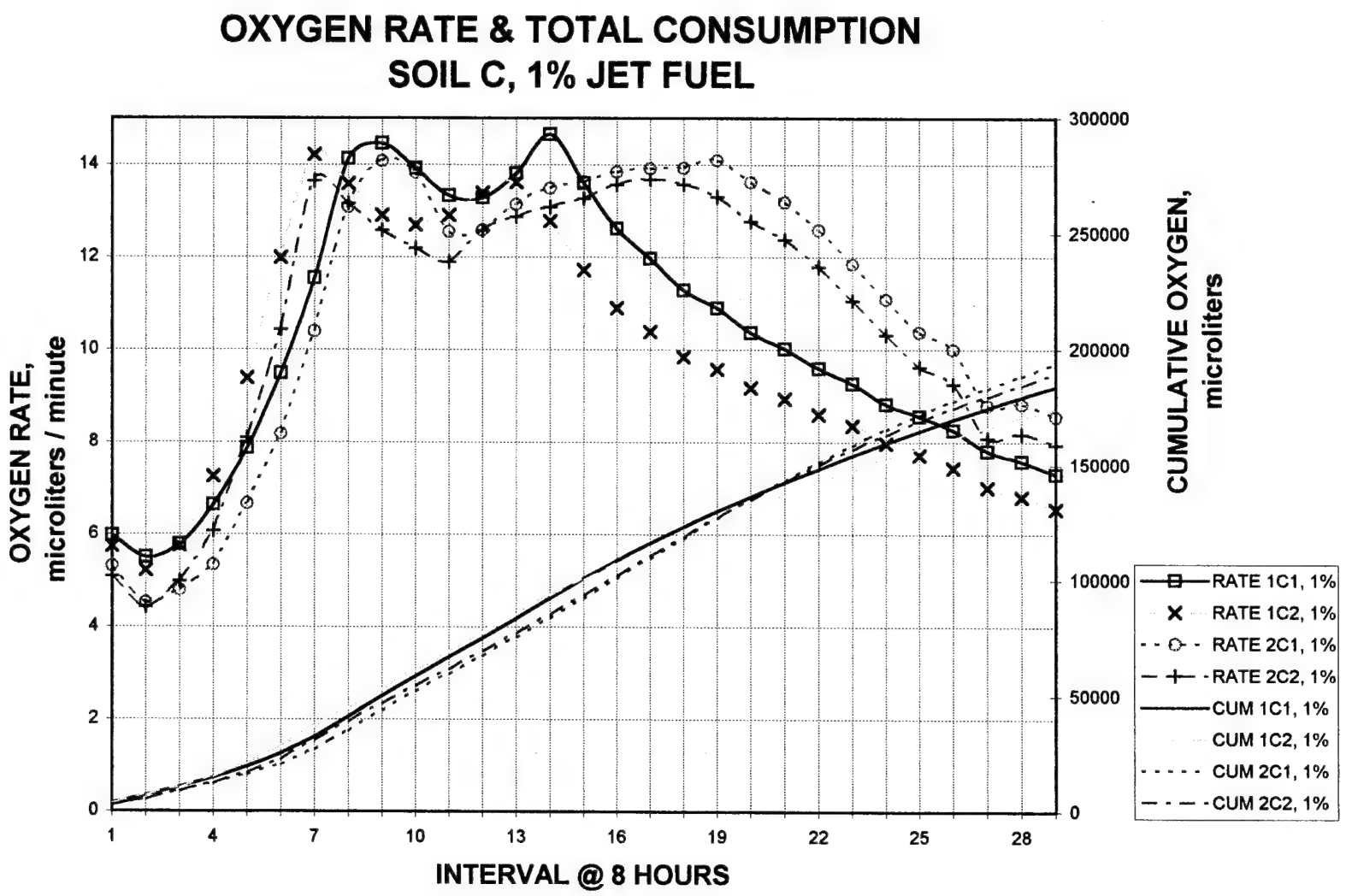

OXYGEN RATE & TOTAL CONSUMPTION
SOIL A, 0.1% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
10
8
6
4
2
0
-2
-4
300000
250000
200000
150000
100000
50000
0
4
7
10
13
16
19
22
25
28
RATE 1A1, 0.1%
RATE 1A2, 0.1%
RATE 2A1, 0.1%
RATE 2A2, 0.1%
CUM 1A1, 0.1%
CUM 1A2, 0.1%
CUM 2A1, 0.1%
CUM 2A2, 0.1%

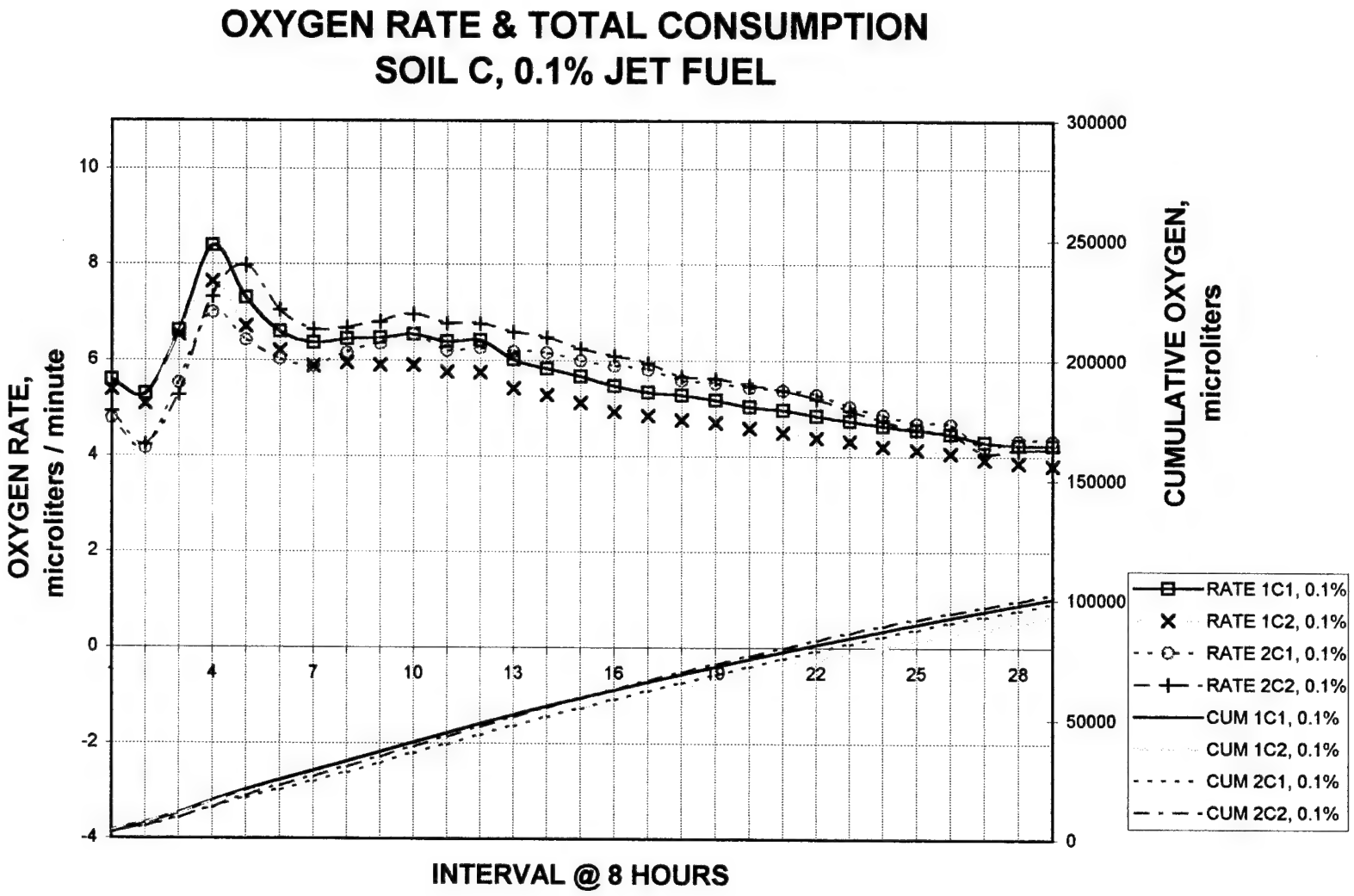

OXYGEN RATE & TOTAL CONSUMPTION
SOIL A, 0% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
10
8
6
4
2
0
-2
-4
300000
250000
200000
150000
100000
50000
0
4
7
10
13
16
19
22
25
28
RATE 1A1, 0%
RATE 1A2, 0%
RATE 2A1, 0%
RATE 2A2, 0%
CUM 1A1, 0%
CUM 1A2, 0%
CUM 2A1, 0%
CUM 2A2, 0%

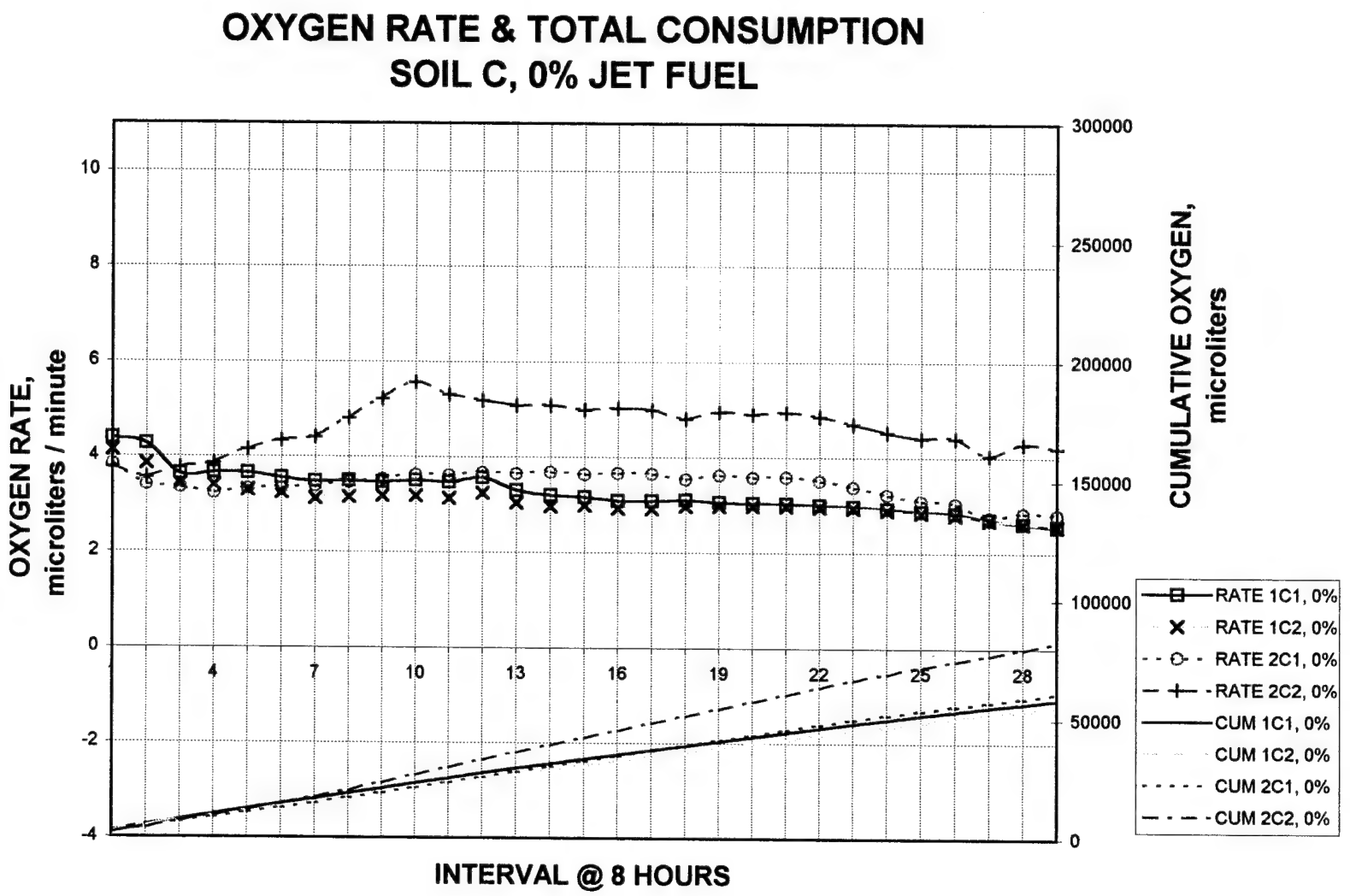

OXYGEN RATE & TOTAL CONSUMPTION
SOIL B, 1% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
10
8
6
4
2
0
-2
-4
300000
250000
200000
150000
100000
50000
0
4
7
10
13
16
19
22
25
28
RATE 1B1, 1%
RATE 1B2, 1%
RATE 2B1, 1%
RATE 2B2, 1%
CUM 1B1, 1%
CUM 1B2, 1%
CUM 2B1, 1%
CUM 2B2, 1%


OXYGEN RATE & TOTAL CONSUMPTION
SOIL B, 0.1% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
10
8
6
4
2
0
-2
-4
300000
250000
200000
150000
100000
50000
0
4
7
10
13
16
19
22
25
28
RATE 1B1, 0.1%
RATE 1B2, 0.1%
RATE 2B1, 0.1%
RATE 2B2, 0.1%
CUM 1B1, 0.1%
CUM 1B2, 0.1%
CUM 2B1, 0.1%
CUM 2B2, 0.1%

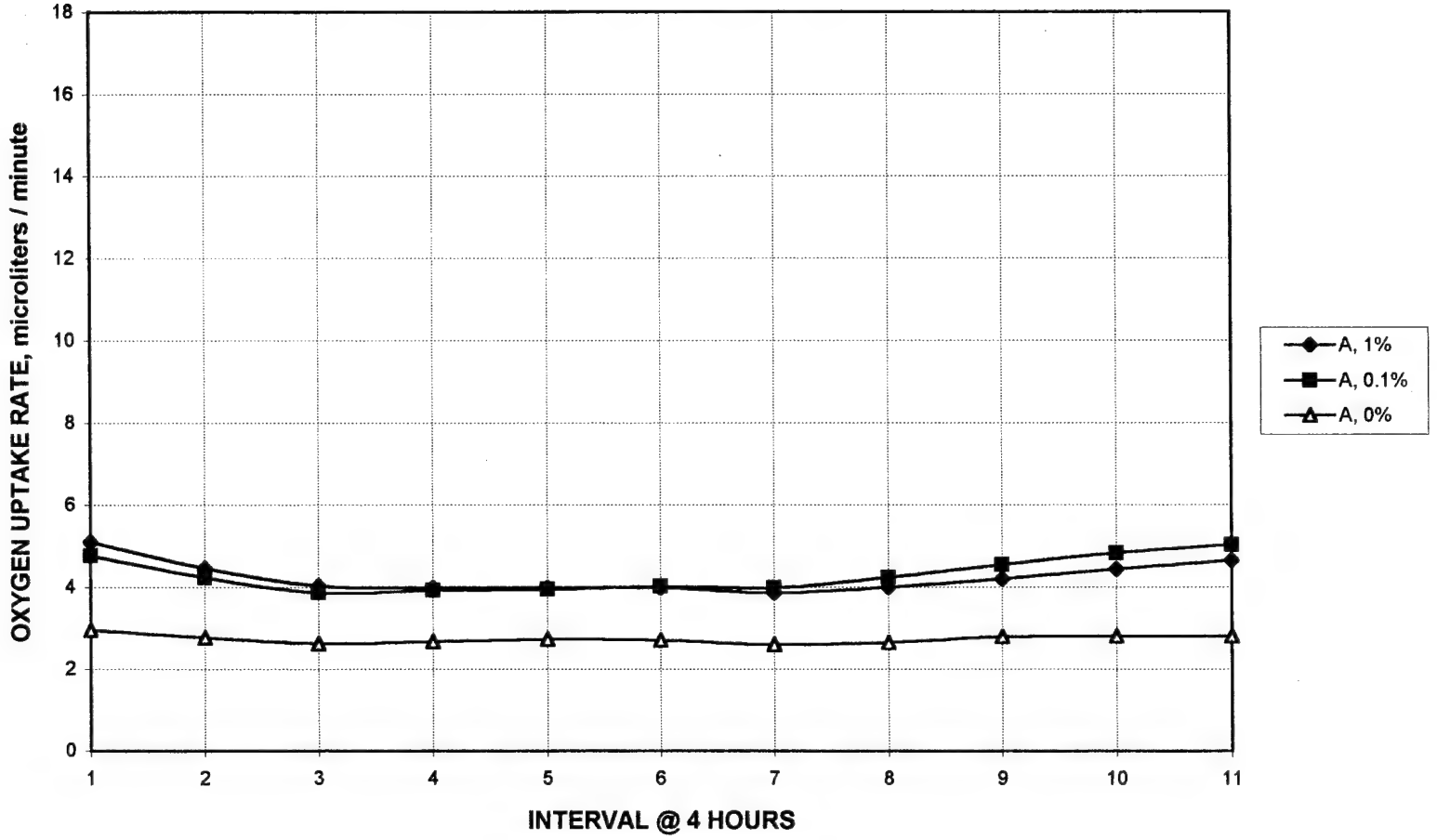

OXYGEN RATE & TOTAL CONSUMPTION
SOIL B, 0% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
10
8
6
4
2
0
-2
-4
300000
250000
200000
150000
100000
50000
0
4
7
10
13
16
19
22
25
28
RATE 1B1, 0%
RATE 1B2, 0%
RATE 2B1, 0%
RATE 2B2, 0%
CUM 1B1, 0%
CUM 1B2, 0%
CUM 2B1, 0%
CUM 2B2, 0%

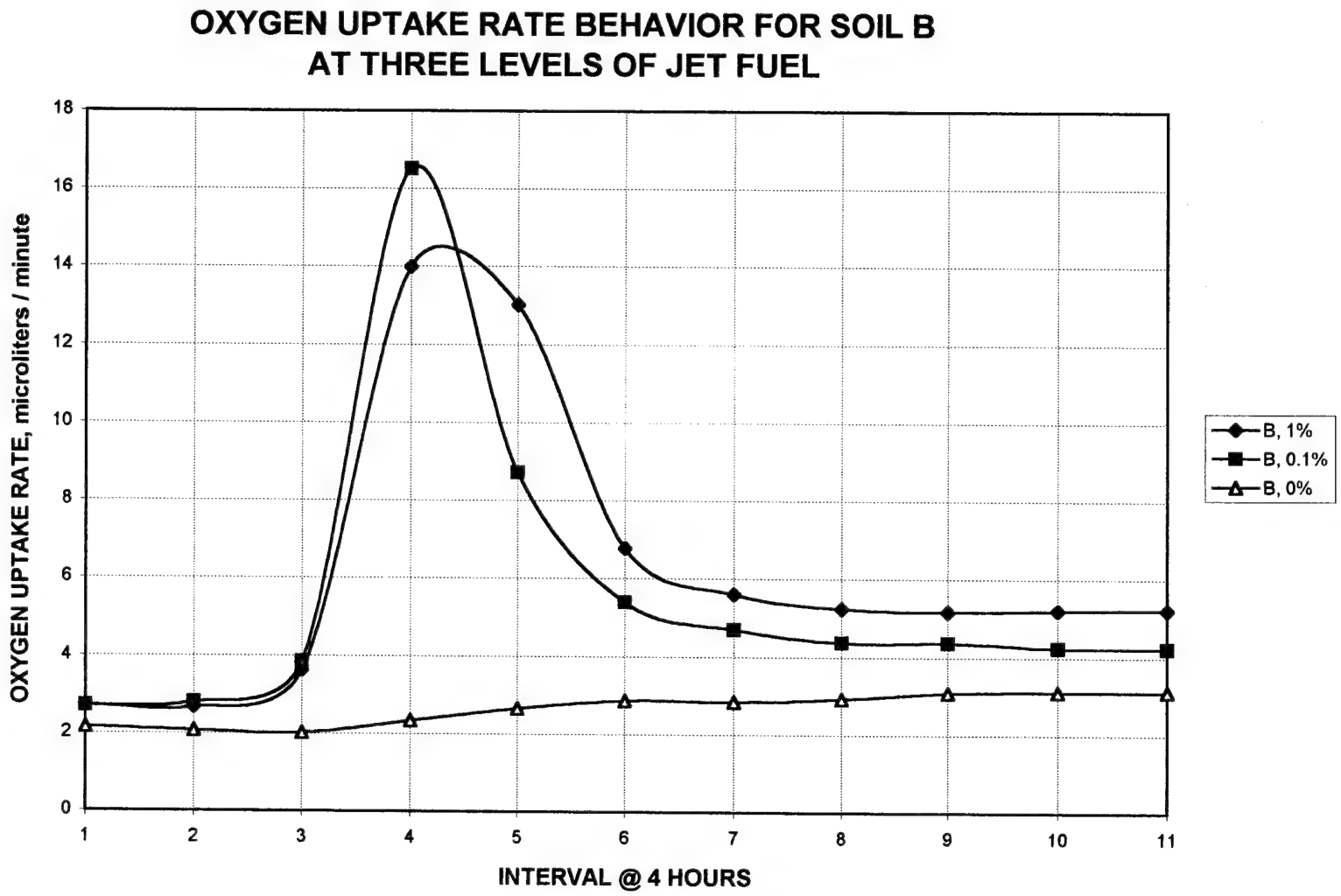

OXYGEN RATE & TOTAL CONSUMPTION
SOIL C, 1% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
0
2
4
6
8
10
12
14
0
50000
100000
150000
200000
250000
300000
1
4
7
10
13
16
19
22
25
28
RATE 1C1, 1%
RATE 1C2, 1%
RATE 2C1, 1%
RATE 2C2, 1%
CUM 1C1, 1%
CUM 1C2, 1%
CUM 2C1, 1%
CUM 2C2, 1%

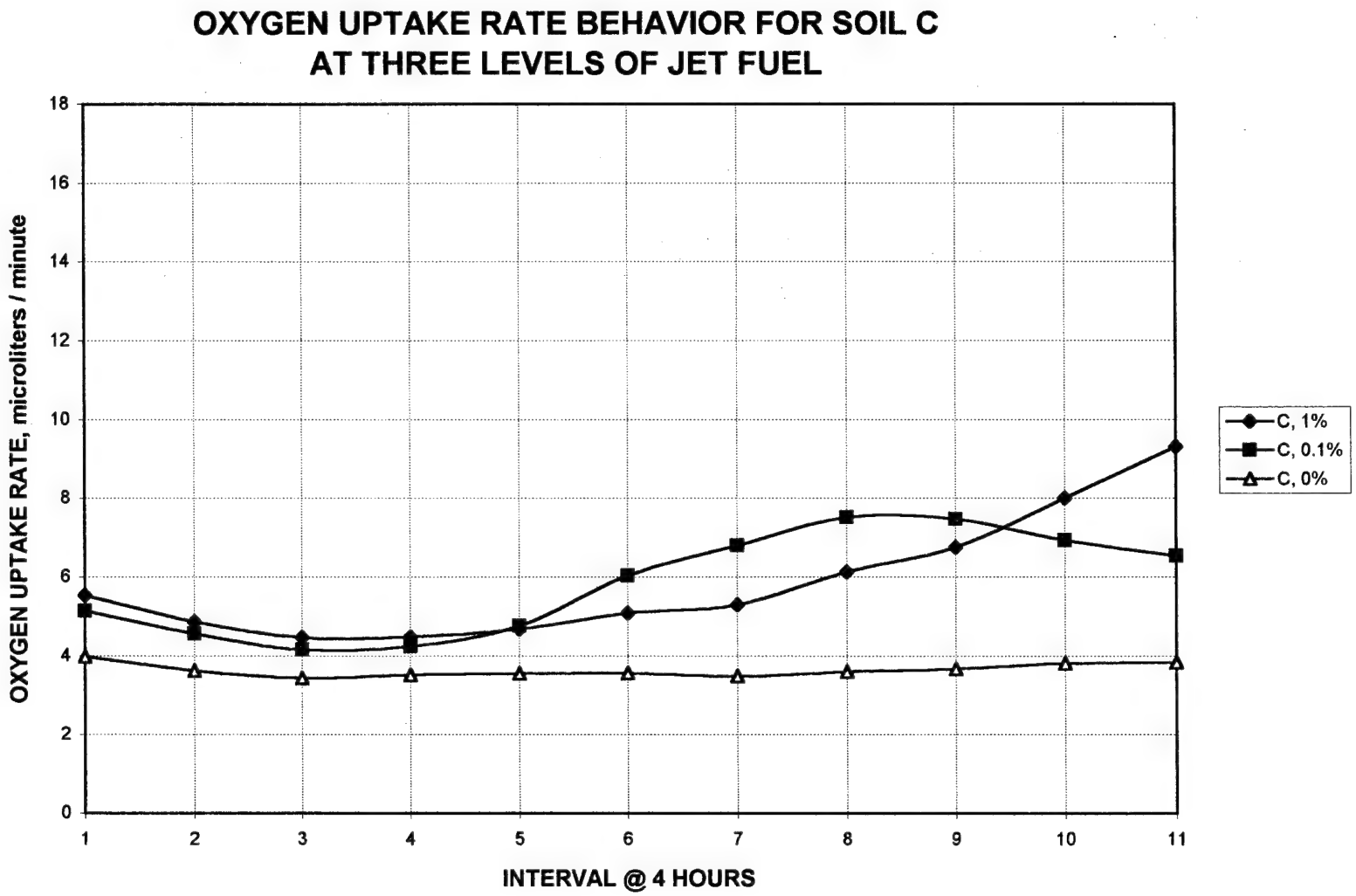

OXYGEN RATE & TOTAL CONSUMPTION
SOIL C, 0.1% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
10
8
6
4
2
0
-2
-4
300000
250000
200000
150000
100000
50000
0
4
7
10
13
16
19
22
25
28
RATE 1C1, 0.1%
RATE 1C2, 0.1%
RATE 2C1, 0.1%
RATE 2C2, 0.1%
CUM 1C1, 0.1%
CUM 1C2, 0.1%
CUM 2C1, 0.1%
CUM 2C2, 0.1%

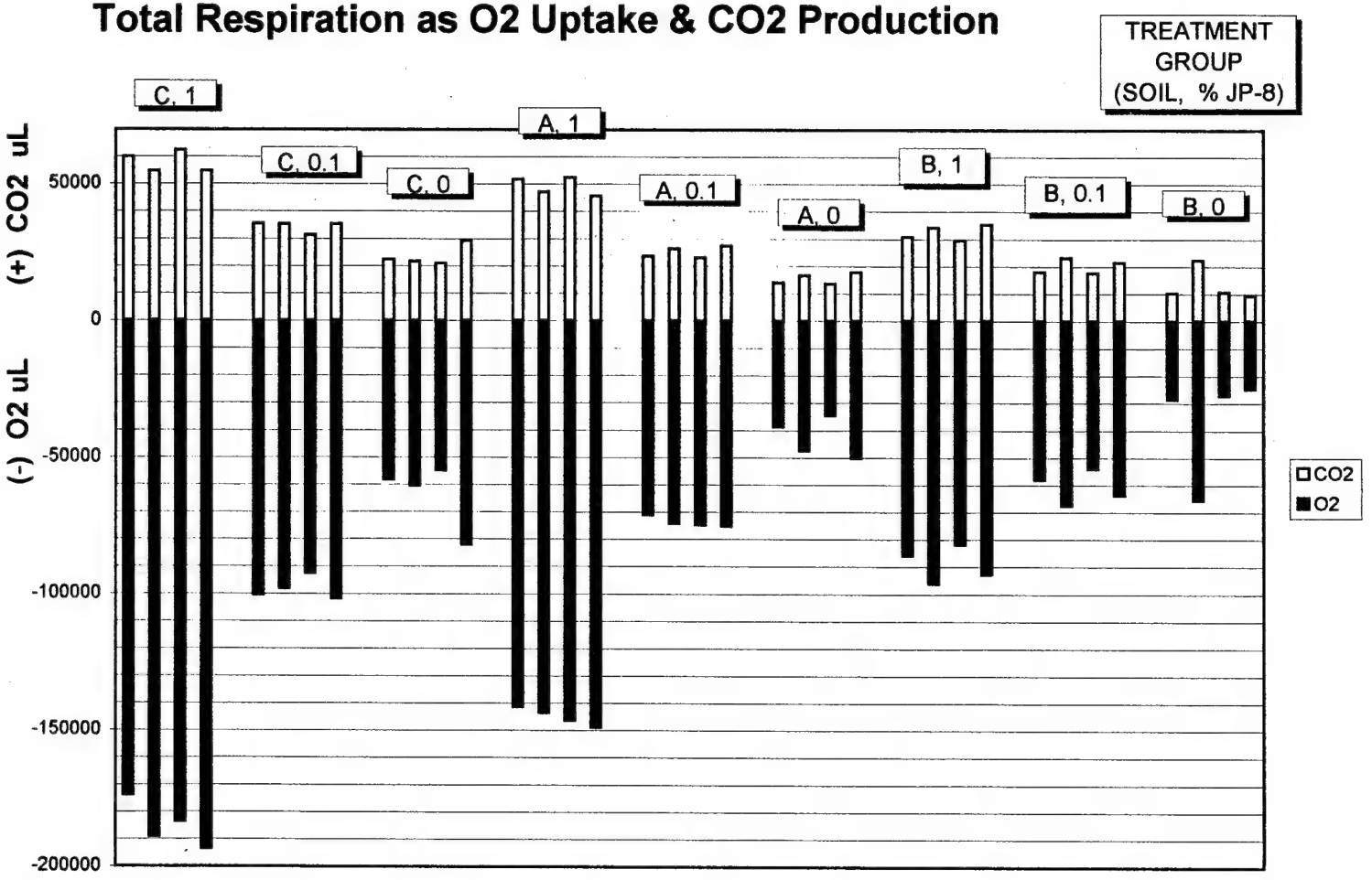

OXYGEN RATE & TOTAL CONSUMPTION
SOIL C, 0% JET FUEL
OXYGEN RATE, microliters / minute
CUMULATIVE OXYGEN, microliters
INTERVAL @ 8 HOURS
10
8
6
4
2
0
-2
-4
300000
250000
200000
150000
100000
50000
0
4
7
10
13
16
19
22
25
28
RATE 1C1, 0%
RATE 1C2, 0%
RATE 2C1, 0%
RATE 2C2, 0%
CUM 1C1, 0%
CUM 1C2, 0%
CUM 2C1, 0%
CUM 2C2, 0%

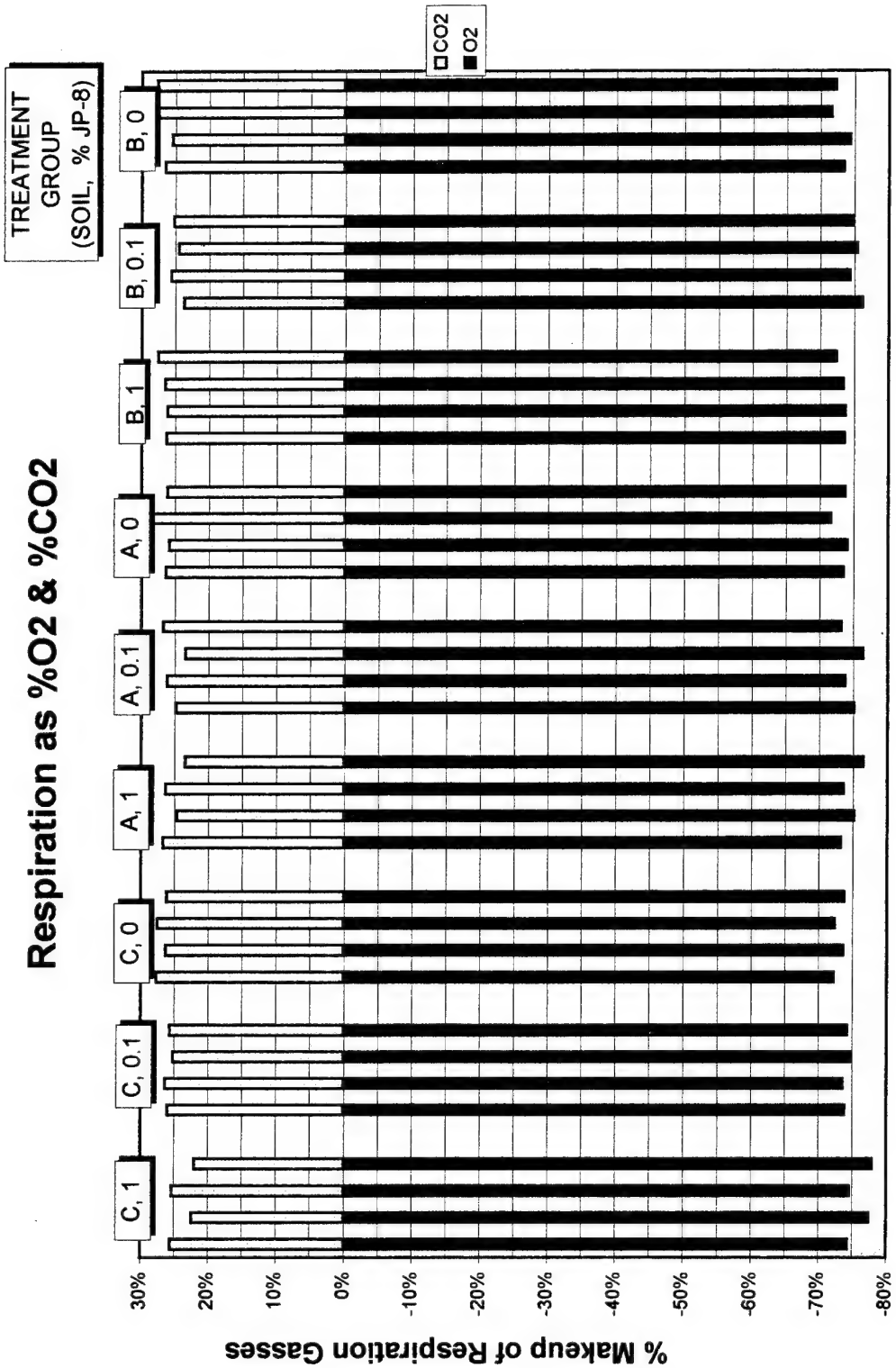
## OXYGEN UPTAKE RATE BEHAVIOR FOR SOIL A AT THREE LEVELS OF JET FUEL




OXYGEN UPTAKE RATE BEHAVIOR FOR SOIL B
AT THREE LEVELS OF JET FUEL
OXYGEN UPTAKE RATE, microliters / minute
0
2
4
6
8
10
12
14
16
18
1
2
3
4
5
6
7
8
9
10
11
INTERVAL @ 4 HOURS
B, 1%
B, 0.1%
B, 0%


OXYGEN UPTAKE RATE BEHAVIOR FOR SOIL C
AT THREE LEVELS OF JET FUEL
OXYGEN UPTAKE RATE, microliters / minute
0
2
4
6
8
10
12
14
16
18
1
2
3
4
5
6
7
8
9
10
11
INTERVAL @ 4 HOURS
C, 1%
C, 0.1%
C, 0%

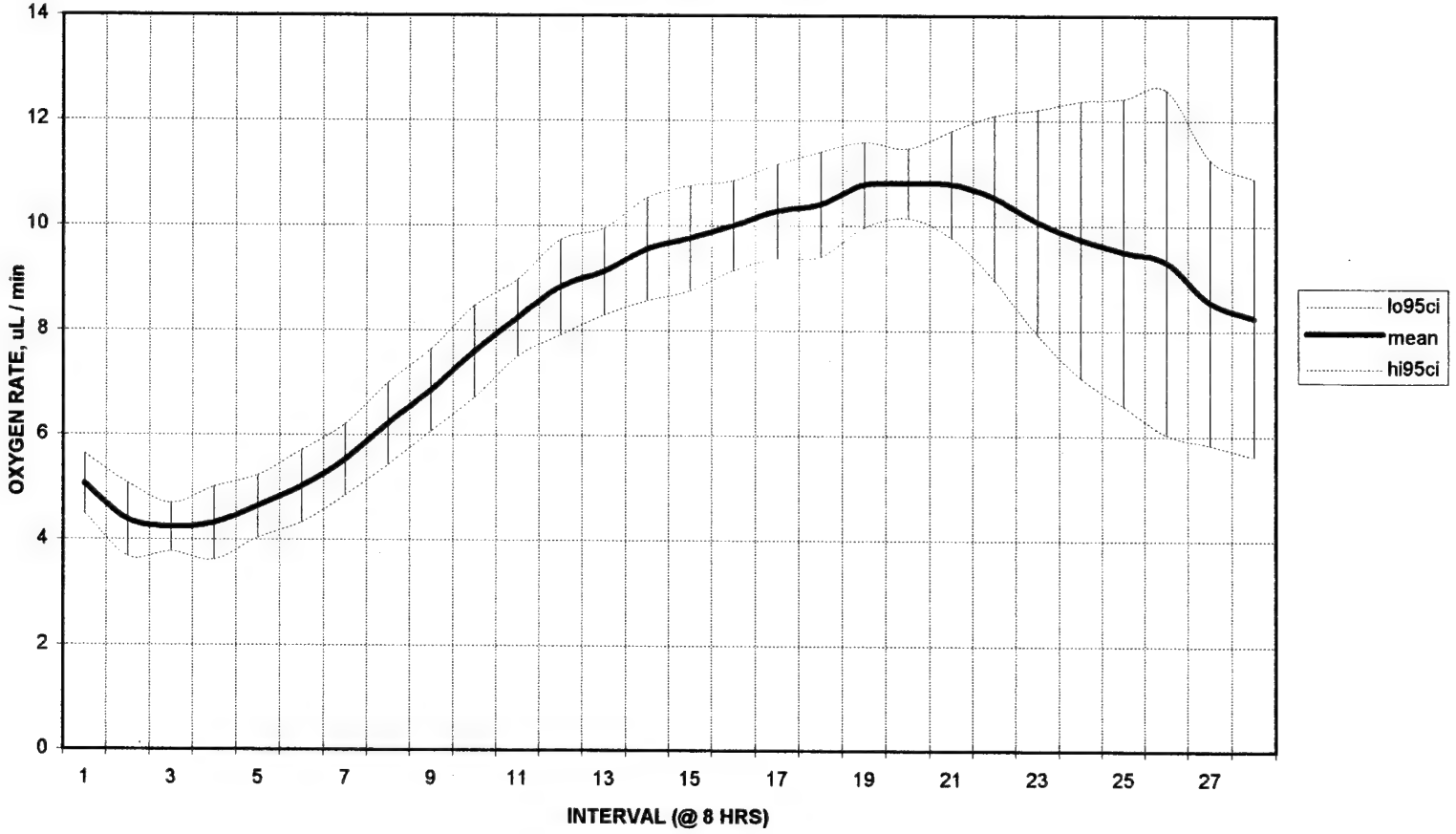

Total Respiration as O2 Uptake & CO2 Production
TREATMENT GROUP (SOIL, % JP-8)
C, 1
C, 0.1
C, 0
A, 1
A, 0.1
A, 0
B, 1
B, 0.1
B, 0
(+) CO2 uL
(-) O2 uL
50000
0
-50000
-100000
-150000
-200000
CO2
O2


Respiration as %O2 & %CO2
TREATMENT GROUP (SOIL, % JP-8)
CO2
O2
C, 1
C, 0.1
C, 0
A, 1
A, 0.1
A, 0
B, 1
B, 0.1
B, 0
30%
20%
10%
0%
-10%
-20%
-30%
-40%
-50%
-60%
-70%
-80%
% Makeup of Respiration Gasses

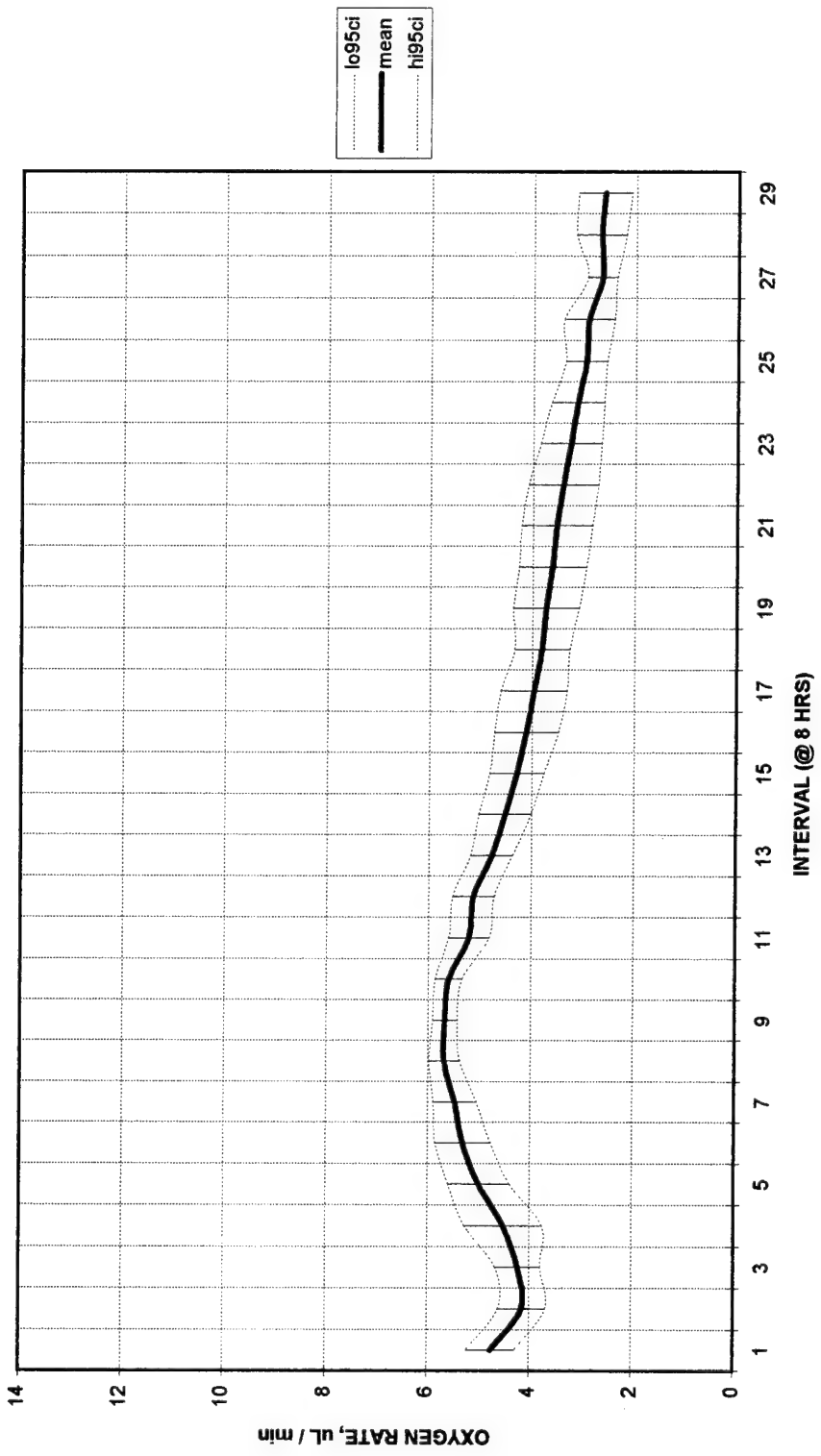
## Appendix F: 95% Confidence Interval & Kinetics Model Curves

The plots of the means of the four replications of each treatment's experimental data with their 95% confidence intervals (computed with the Statistix™ package) follow. The output curves of the 3/2 Kinetics model for hydrocarbon mineralization follow these.

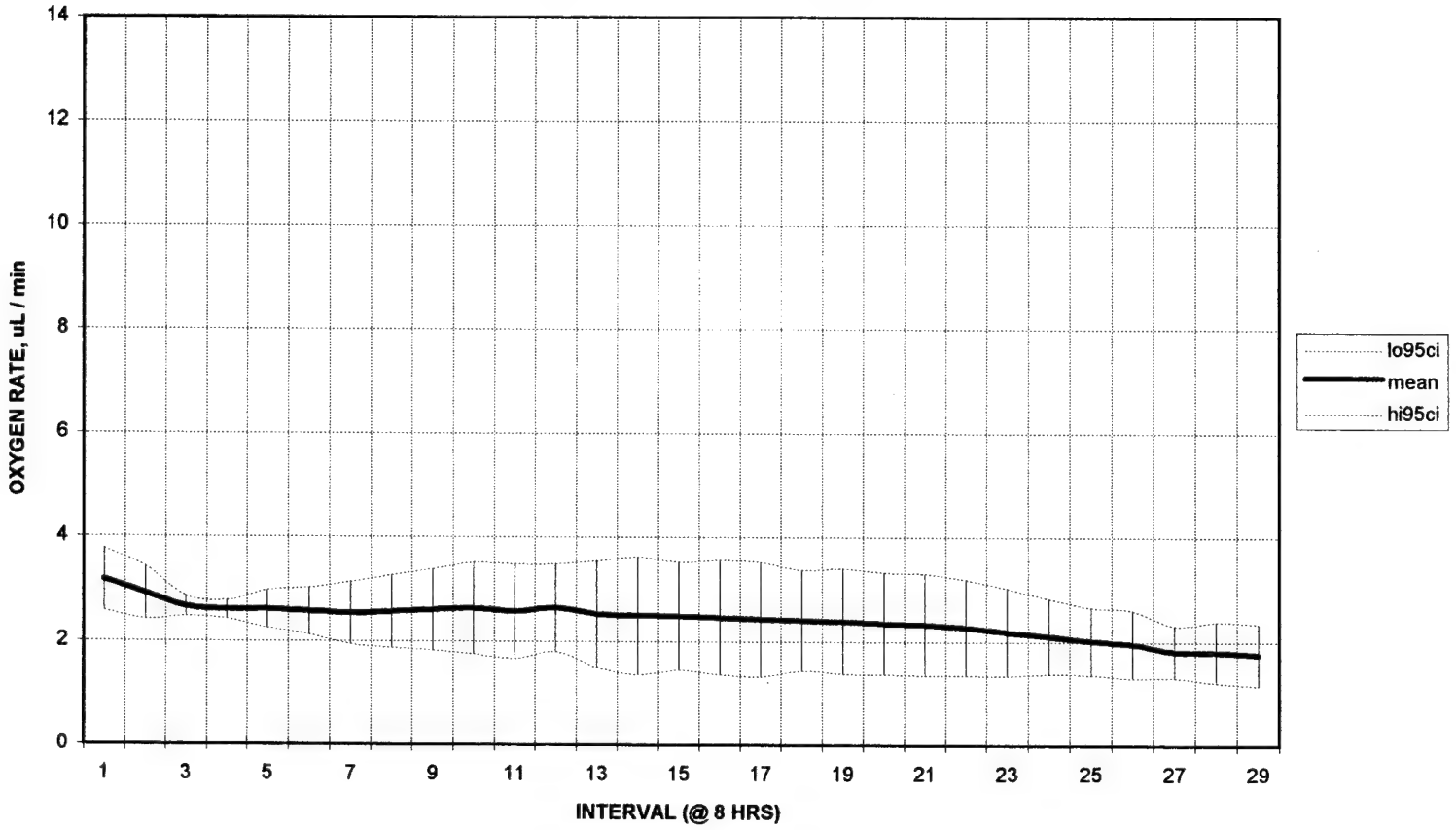
## 95% Confidence Interval for Oxygen Consumption Data
## SOIL A; 1% JP-8



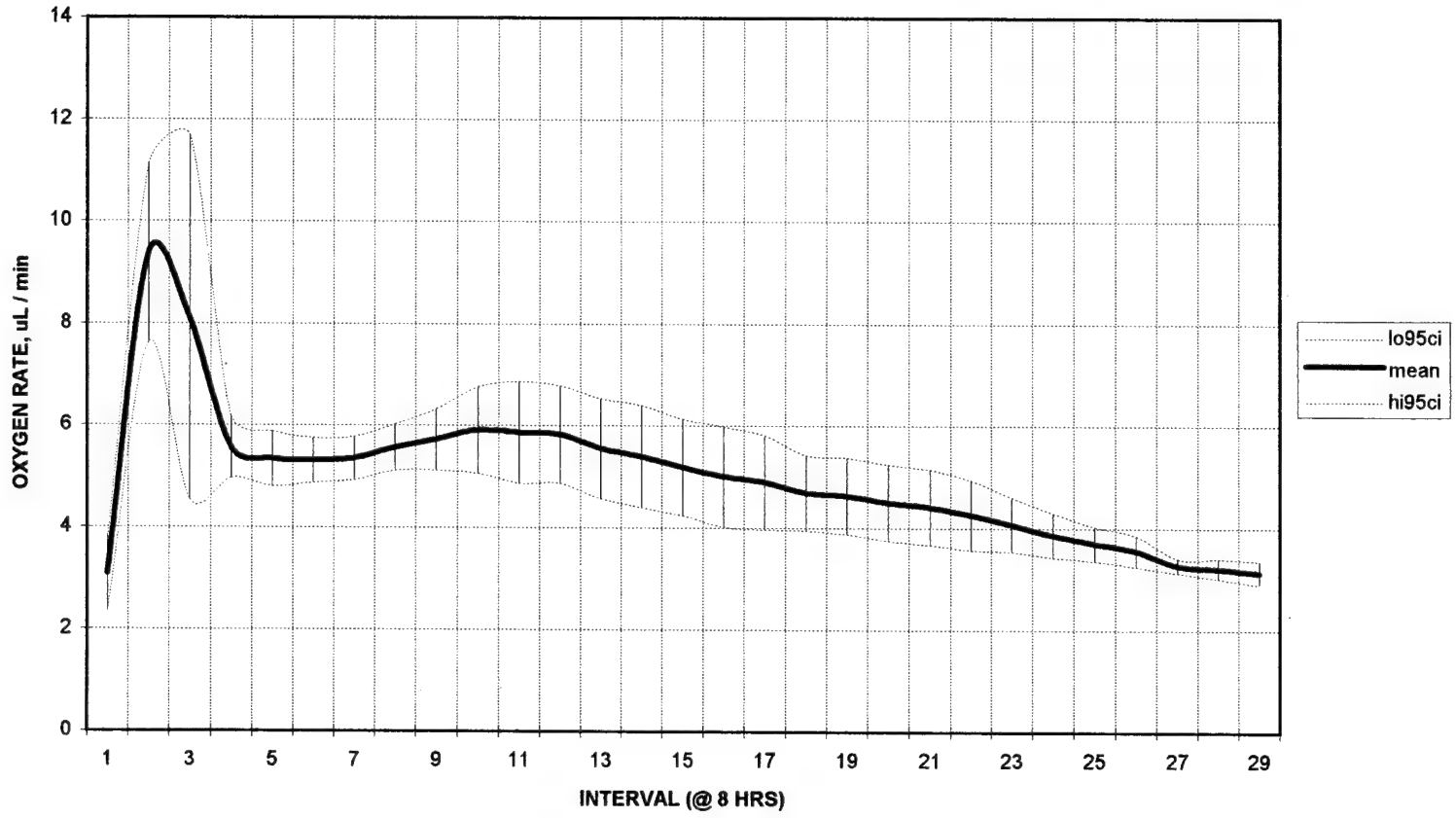
# 95% Confidence Interval for Oxygen Consumption Data
## SOIL A; 0.1% JP-8

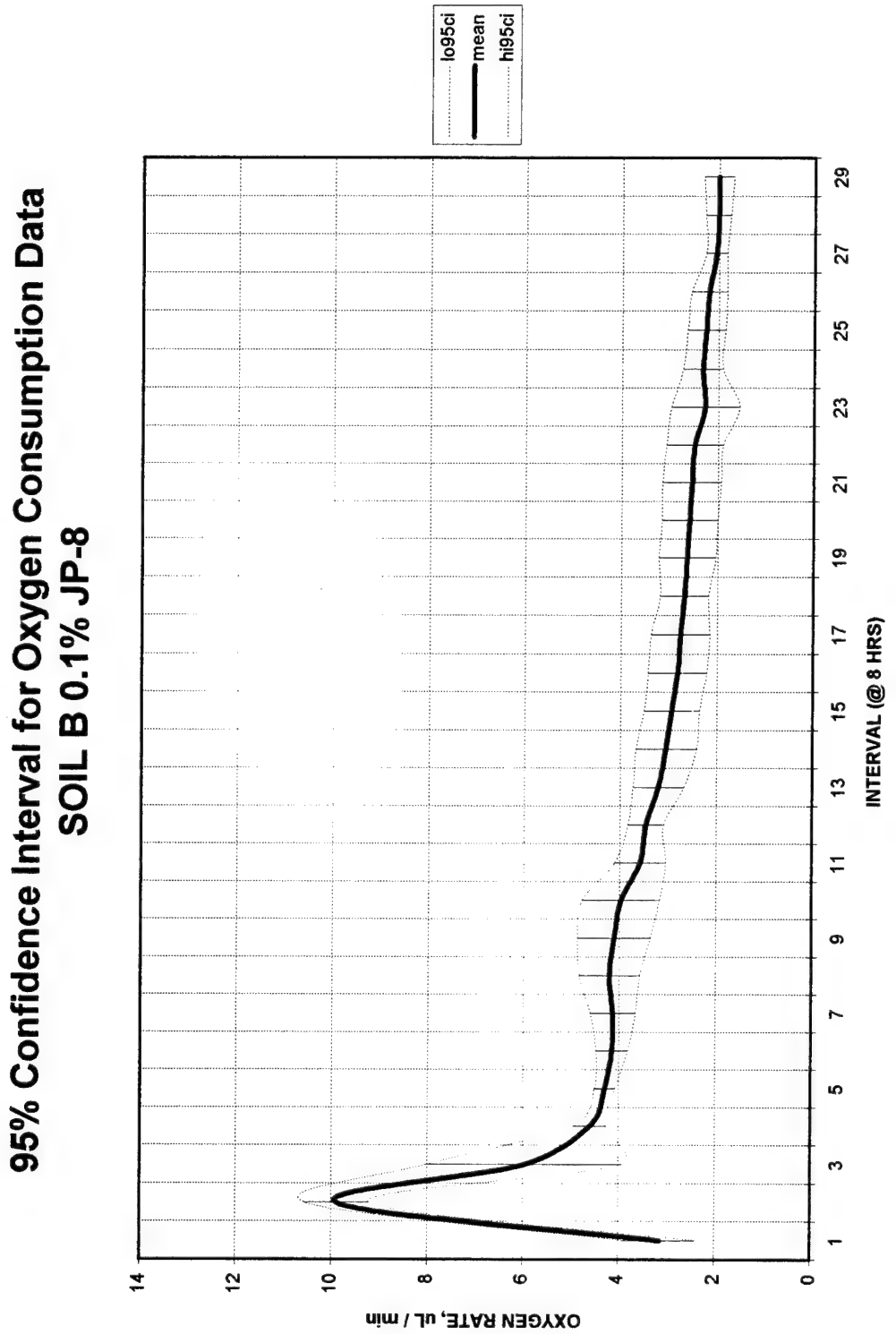


# 95% Confidence Interval for Oxygen Consumption Data
# SOIL A; 0% JP-8

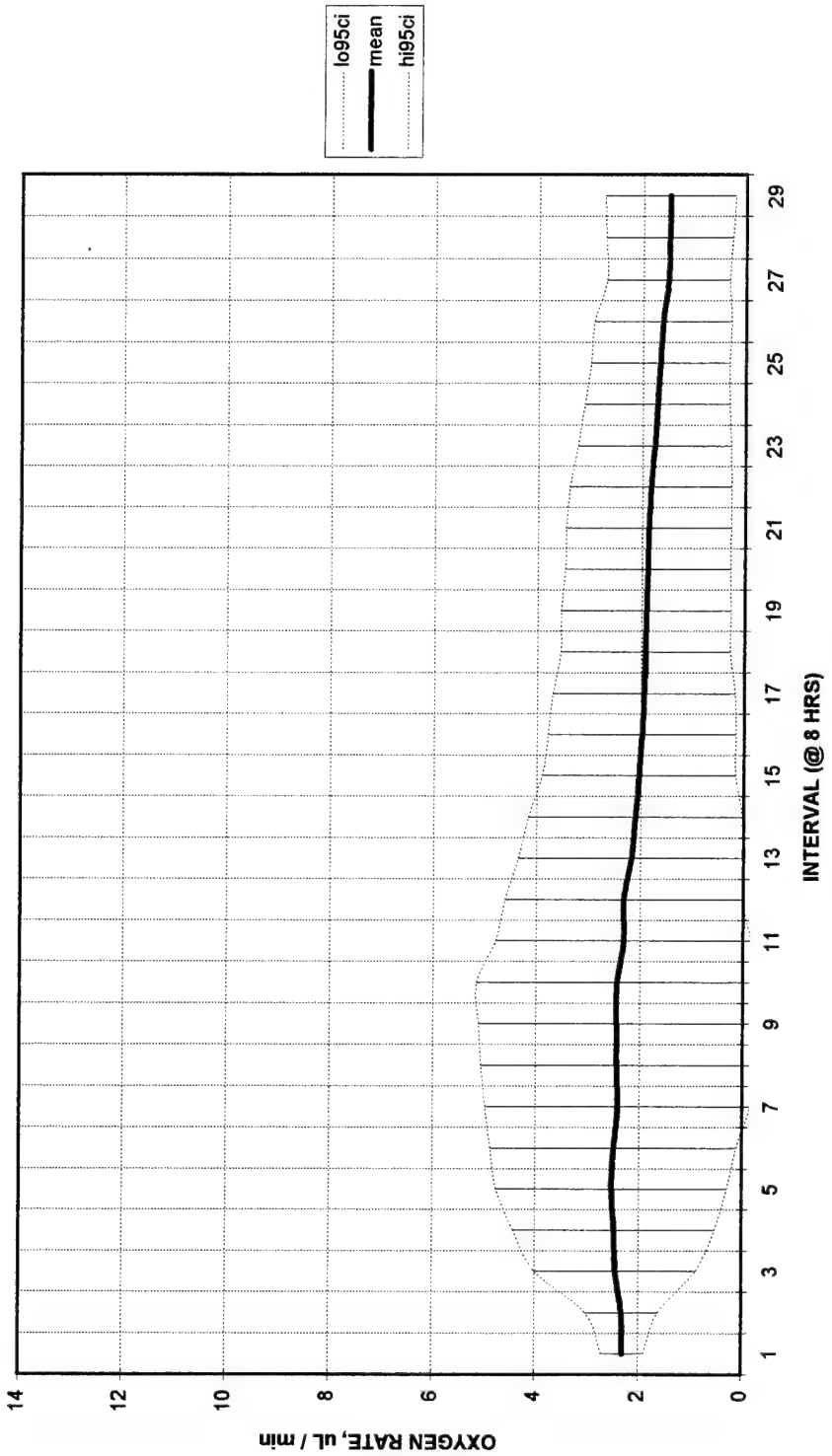


# 95% Confidence Interval for Oxygen Consumption Data
# SOIL B; 1% JP-8

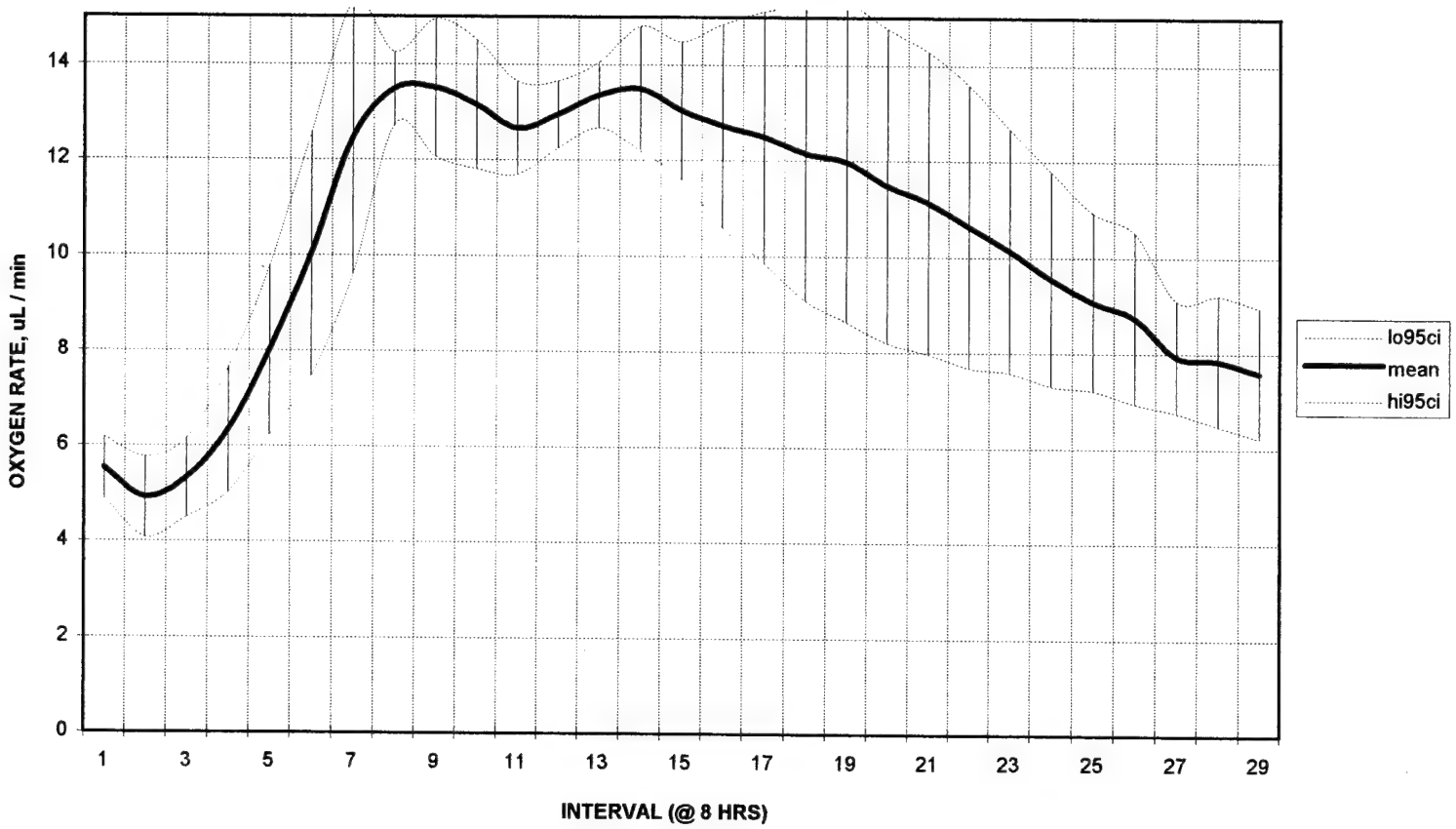



95% Confidence Interval for Oxygen Consumption Data
SOIL B 0.1% JP-8
lo95ci
mean
hi95ci
OXYGEN RATE, uL / min
0
2
4
6
8
10
12
14
INTERVAL (@ 8 HRS)
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29

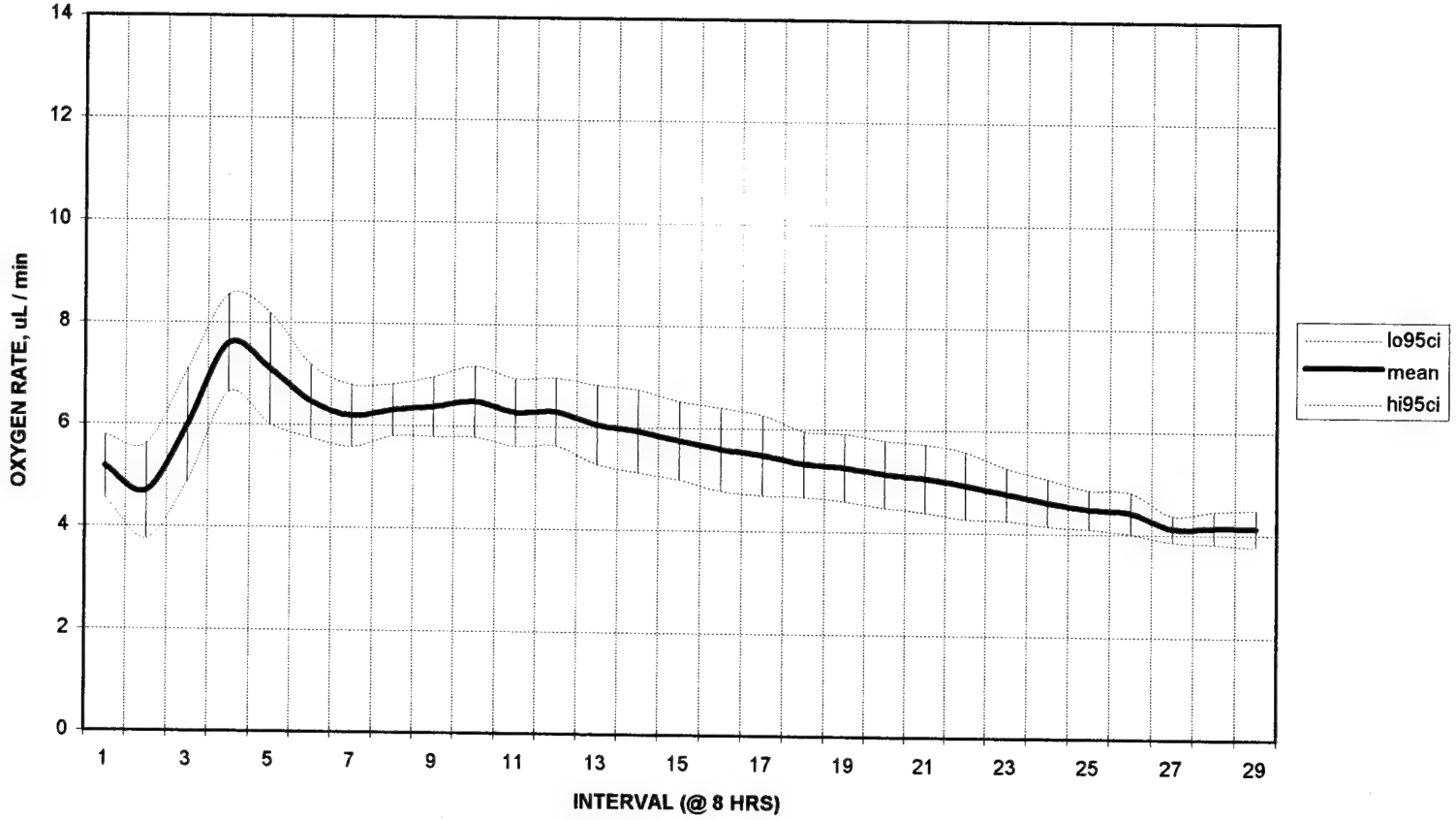
# 95% Confidence Interval for Oxygen Consumption Data
## SOIL B; 0% JP-8

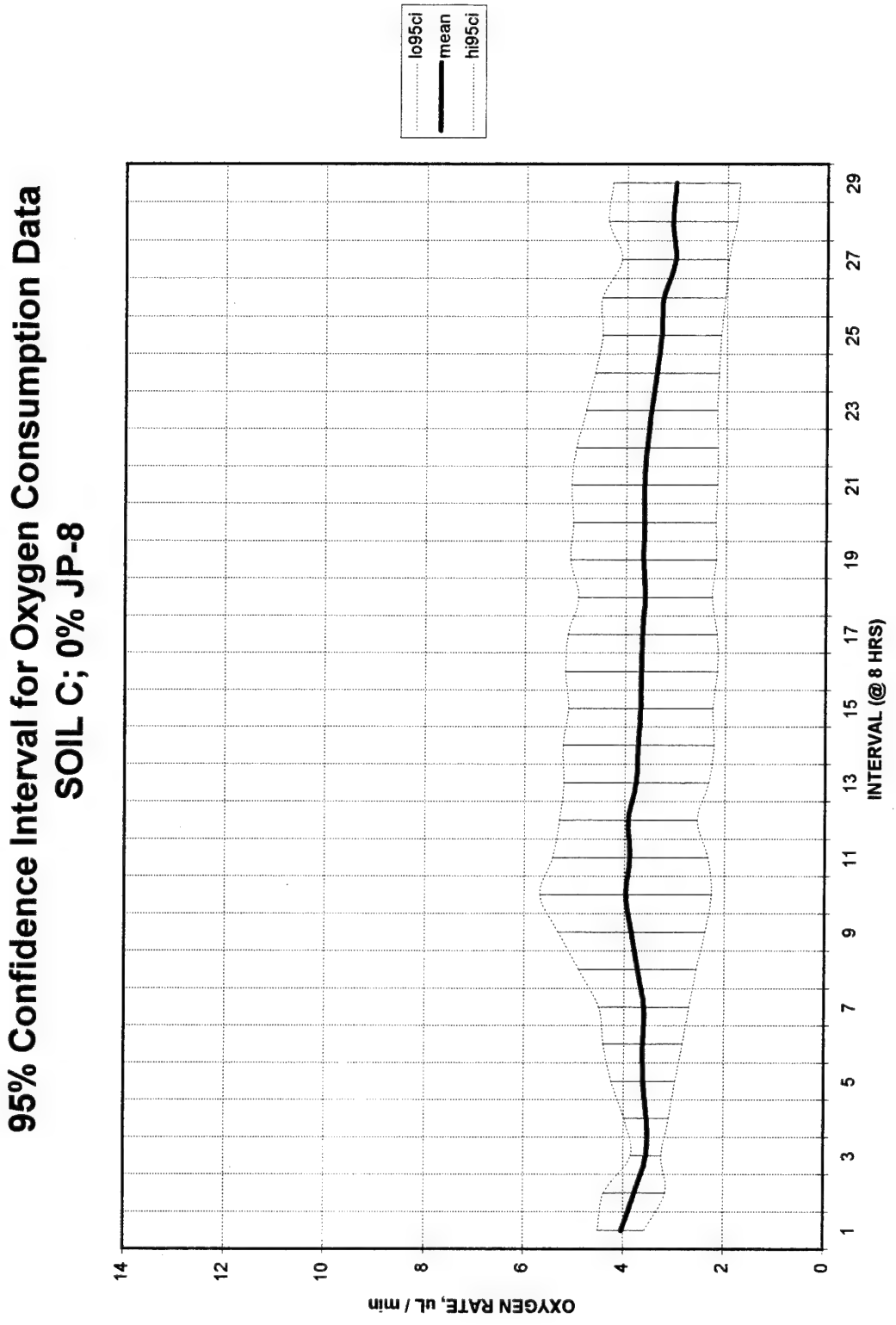


# 95% Confidence Interval for Oxygen Consumption Data
# SOIL C; 1% JP-8



# 95% Confidence Interval for Oxygen Consumption Data
# SOIL C; 0.1% JP-8




95% Confidence Interval for Oxygen Consumption Data
SOIL C; 0% JP-8
lo95ci
mean
hi95ci
OXYGEN RATE, uL / min
0
2
4
6
8
10
12
14
INTERVAL (@ 8 HRS)
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29

# 3/2 ORDER MINERALIZATION MODEL

P = percentage of compound mineralized at time, T

$P_0$ = percentage of compound converted to $CO_2$ during first order metabolism $P_0 := 25$

$k_0$ = zero order rate constant (% day $^{-1}$) $k0 := 1$

$k_1$ = proportionality rate constant (day $^{-1}$) $k1 := .01$

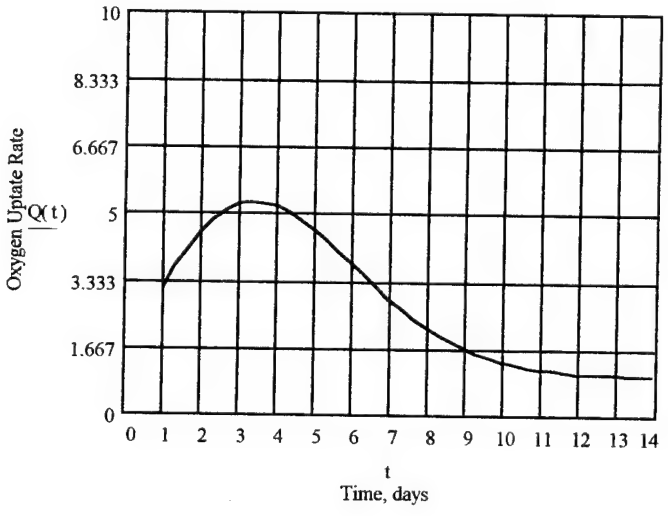
$k_2$ = linear growth rate term $k2 := .08$

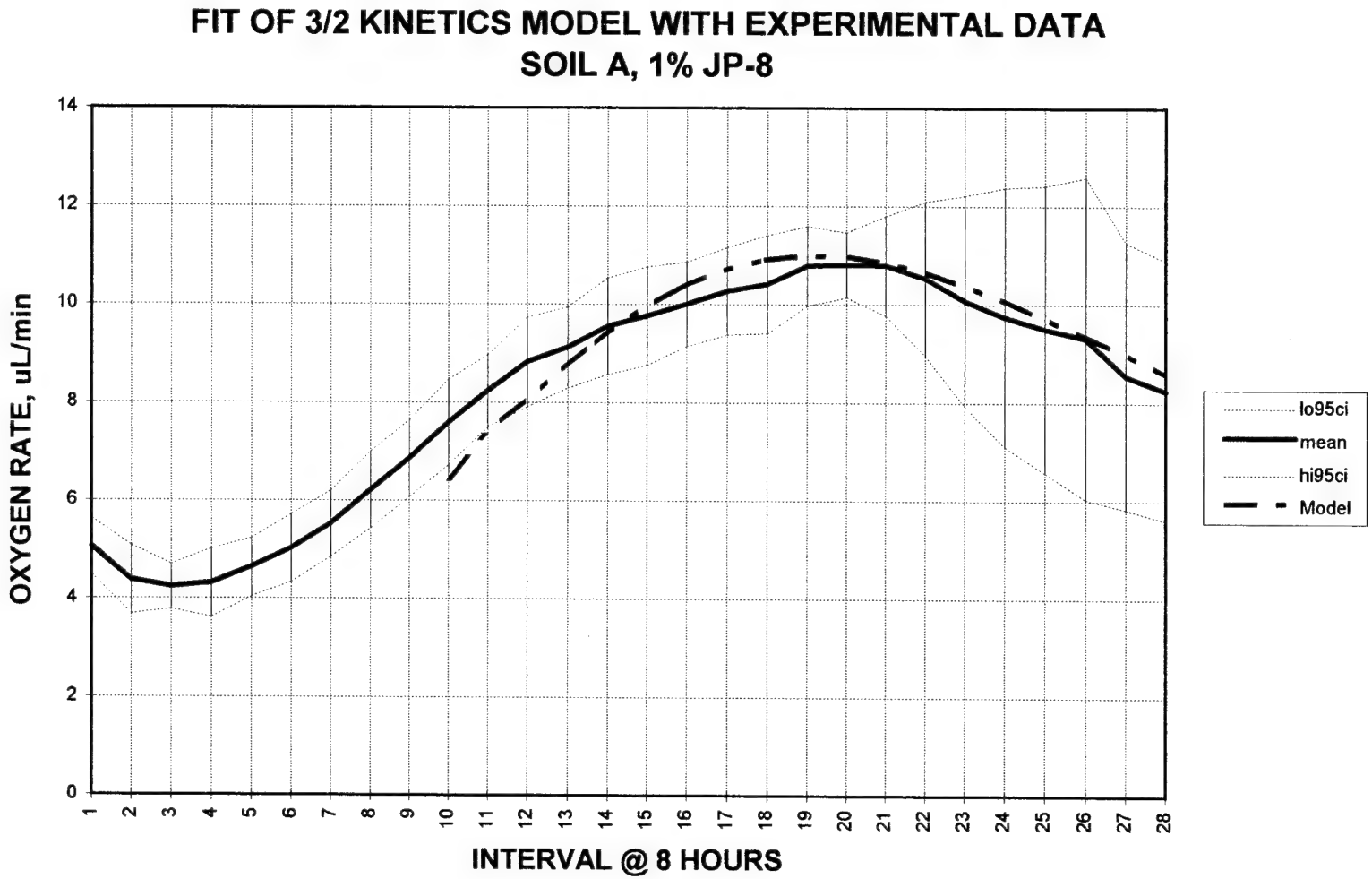
t = time interval for plotting $t := 1, 1.33.. 14$

The model, in terms of percentage of compound $P(t) := P_0 \cdot \left[ 1 - e^{\left[ -k1 \cdot t - \frac{k2 \cdot \left[ (t)^2 \right]}{2} \right]} \right] + k0 \cdot t$

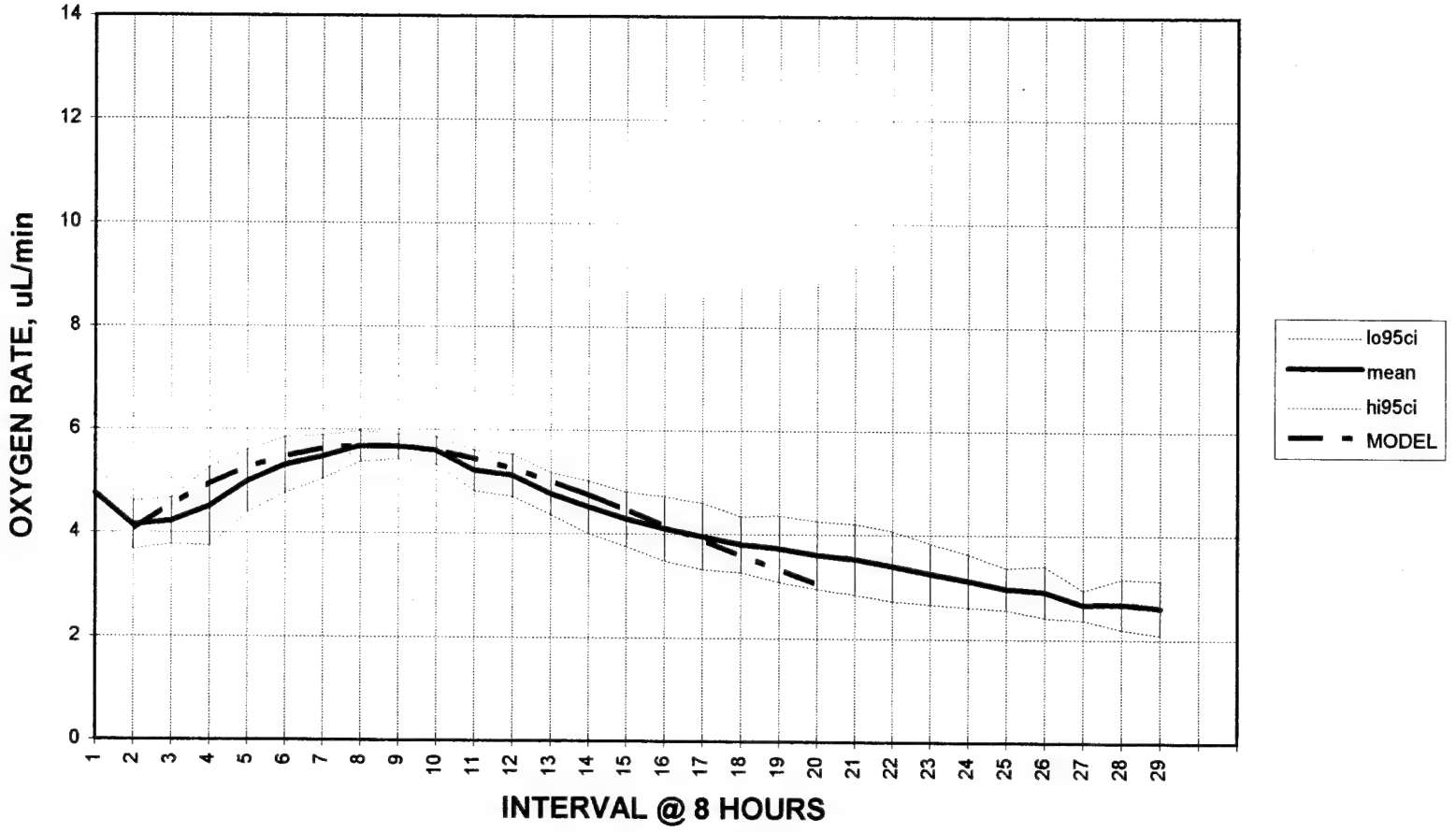
The first derivative of the model, to show rates $Q(t) := \frac{d}{dt} P(t)$

The plot of rate vs time:




FIT OF 3/2 KINETICS MODEL WITH EXPERIMENTAL DATA
SOIL A, 1% JP-8
OXYGEN RATE, uL/min
INTERVAL @ 8 HOURS
lo95ci
mean
hi95ci
Model

## FIT OF 3/2 KINETICS MODEL WITH EXPERIMENTAL DATA
## SOIL A, 0.1% JP-8



## Appendix G: Respiration Ratio Data and $CO_2$ Accounting

The following pages contain the sample calculations used to analyze the respiration ratios (actual and predicted) and also the carbon dioxide accounting. Also shown are the sample calculations for the quantification of the jet fuel biodegraded predicted by the oxygen uptake. These sample calculations back up Tables 4.3 and 4.5.

# Sample calculations for Table 4.3

| % Clay, Fuel Level | Mean Oxygen Consumed, ul | Mean Oxygen Consumed*, L | Mean Oxygen Cons., moles | Dry soil kg | Resp Rate* ul/min/kg | Total Fuel Cons***,ml | Orig Fuel Added, ml | % Lost to Biodeg | HC Degr Rate ml/day/kg |
|---|---|---|---|---|---|---|---|---|---|
| 29, 1% | 185220.9475 | 0.184296948 | 0.007587609 | 0.0862 | 154.36314 | 0.0571826 | 1.3725744 | 4.166081 | 0.068600903 |
| 29, 0.1% | 98425.82 | 0.09750182 | 0.004014205 | 0.0862 | 82.028079 | 0.0161942 | 0.1372574 | 11.79842 | 0.019427901 |
| 29, 0% | 64133.6875 | 0.063209688 | 0.002602378 | 0.0862 | 53.449016 | | | | |
| 16, 1% | 145246.3675 | 0.144322368 | 0.005941833 | 0.0798 | 130.75646 | 0.0483411 | 1.2666596 | 3.816427 | 0.062645154 |
| 16, 0.1% | 74015.1775 | 0.073091178 | 0.003009205 | 0.0798 | 66.631357 | 0.0147027 | 0.126666 | 11.60749 | 0.019053238 |
| 16. 0% | 42881.3125 | 0.041957313 | 0.001727406 | 0.0798 | 38.603434 | | | | |
| 6, 1% | 89354.7875 | 0.088430788 | 0.003640745 | 0.0845 | 75.966459 | 0.0249222 | 1.2537038 | 1.987883 | 0.030500199 |
| 6, 0.1% | 60995.3375 | 0.060071338 | 0.002473171 | 0.0845 | 51.856201 | 0.0115296 | 0.1253704 | 9.196455 | 0.014110169 |
| 6, 0% | 36580.7 | 0.0356567 | 0.001468006 | 0.0845 | 31.099691 | | | | |
| empty | 924 | | | | | | | | |

Data from the respirometer.

Converting to liters, then reducing by the background level from the empty microcosm.

Solving the perfect gas law for n, number of moles, using standard pressure (1 atm) and ambient temperature (296°K) and R = .082058 L-atm/°K-mol.

Dry weight of soil in each microcosm.

Converting units, using total time in microcosms (232 hours) and weight of soil.

Using total oxygen due do fuel (reducing total by amount in zero fuel treatment) with total time and weight of soil and converting from moles to grams using MW of $C_{12}H_{26}$, then converting to volume.

Converting units.

## SAMPLE CALCULATIONS

## QUANTIFICATION OF HYDROCARBON BIODEGRADATION FROM RESPIROMETER DATA (OXYGEN CONSUMPTION)

Conversion from ul of oxygen consumed to ml of hydrocarbon consum and intermediate steps, using first row of Table 4.3 as the example.

$v := 185221$ microliters of oxygen consumed in treatment A, 1%

$v_{act} := v - 924$ adjusting for background $O_2$ readings in empty microcosms

$V := \frac{v_{act}}{1000000}$ converting from microliters to liters

$P := 1$ standard atmospheric pressure, atm

$t := 23$ ambient temperature of the experiments

$T := 273 + t$ converting to degrees Kelvin

$R := .082058$ the gas constant, L-atm / °K-mol

$n := \frac{P \cdot V}{R \cdot T}$ the perfect gas law

$n = 0.00759$ **the number of moles of oxygen consumed**

$intv := 8$ sampling interval, one sample per 8 hours

$time := 29 \cdot intv \cdot 60$ total time of the experiment (min) for 29 intervals

$soil := .086$ weight of soil, kg

$$resp_{rate} := \frac{\frac{v}{time}}{soil}$$

$resp_{rate} = 154.7$ **respiration rate, ul / min / kg dry soil**

$V_{0\%} := .0632$ background respiration of soil without jet fuel

$V_{hc} := V - V_{0\%}$ respiration attributable to hydrocarbon

$n_{hc} := \frac{P \cdot V_{hc}}{R \cdot T}$ moles of O2 attributable to hydrocarbon respiration

$ratio := 18.5$ number of moles of $O_2$ to mineralize 1 mole of $C_{12}H_{26}$

$MW := 169.762$ molecular weight of $C_{12}H_{26}$, gm/mol

$spgr_{hc} := 1.25$ number of milliliters per gram of JP8

$hc := \frac{n_{hc}}{ratio} \cdot MW \cdot spgr_{hc}$

$hc = 0.0572$ **volume of fuel consumed, ml**

$hc_{orig} := 1.3725$ original volume of fuel added, ml

$percent_{lost} := \frac{hc \cdot 100}{hc_{orig}}$

$percent_{lost} = 4.17$ **% fuel lost to biodegradation**

$days := \frac{time}{60 \cdot 24}$ number of days experiment ran

$degrade_{rate} := \frac{\frac{hc}{days}}{soil}$

$degrade_{rate} = 0.0688$ **hydrocarbon degradation rate, ml /day / kg soil**

# Sample Calculations for Table 4.5

| Treatment, Soil, % JP8 | Soil Clay Content, % | Actual Oxygen Cons, ul | Pred. O2:CO2 Ratio | Pred. Total CO2*, ul | Actual CO2 Prod., ul | Act. CO2 Due to Biodeg., ul | Act. O2:CO2 Ratio** | Pred. CO2 Lost to Biomass***, ul | CO2 Unaccounted for, ul | CO2 Unacct. % |
|---|---|---|---|---|---|---|---|---|---|---|
| C, 1 | 29 | 185221 | 1.5417 | 78542 | 56569 | 33563 | 3.6077 | 19635 | 25343 | 32.3 |
| C, 0.1 | 29 | 98426 | 1.5417 | 22244 | 33526 | 10521 | 3.2596 | 5561 | 6162 | 27.7 |
| C, 0 | 29 | 64133 | | | 23005 | | 2.7878 | | | |
| A, 1 | 16 | 145246 | 1.5417 | 66398 | 47912 | 32690 | 3.1314 | 16599 | 17108 | 25.8 |
| A, 0.1 | 16 | 74015 | 1.5417 | 20195 | 24702 | 9480 | 3.2841 | 5049 | 5666 | 28.1 |
| A, 0 | 16 | 42881 | | | 15222 | | 2.8171 | | | |
| B, 1 | 6 | 89355 | 1.5417 | 34231 | 31845 | 18841 | 2.8010 | 8558 | 6832 | 20.0 |
| B, 0.1 | 6 | 60995 | 1.5417 | 15836 | 19778 | 6774 | 3.6044 | 3959 | 5104 | 32.2 |
| B, 0 | 6 | 36581 | | | 13004 | | 2.8130 | | | |

Data from the respirometer.

Calculated from oxygen consumption and the ratio.

Data from the respirometer.

Reduced by background level in zero fuel microcosm.

Calculated from respirometer data.

Theorized to be 25% of total carbon used.

Predicted total CO2 less that lost to biomass and to biodegradation.

# SAMPLE CALCULATIONS

## RESPIRATION RATIOS AND CARBON DIOXIDE ACCOUNTING

Calculation of $O_2$ : $CO_2$ respiration ration from respirometer data and from theory, and quantifying $CO_2$ production and theoretical sources and sinks, using first row of Table 4.5 as the example.

$v_O := 185221$ microliters of oxygen consumed in treatment A, 1%

$v_{Oact} := v_O - 64133$ adjusting for background $O_2$ readings in empty microcosms

$ratio_{co2} := 1.5417$ predicted ratio of $O_2$ to $CO_2$ from complete $C_{12}H_{26}$ mineralization

$$v_{co2} := \frac{v_{Oact}}{ratio_{co2}}$$

$v_{co2} = 78542$ **predicted total $CO_2$ produced**

$v_{1\%} := 56569$ actual $CO_2$ produced in C, 1% microcosm, microliters

$v_{0\%} := 23005$ actual $CO_2$ produced in background microcosm (C, )%), µl

$$ratio_{act} := \frac{v_{Oact}}{v_{1\%} - v_{0\%}}$$

$ratio_{act} = 3.6077$ **actual $O_2$ : $CO_2$ ratio**

$biomass := .25 \cdot v_{co2}$

$biomass = 19635$ **predicted $CO_2$ loss to formation of biomass, µl**

$unaccounted := v_{co2} - \left[ biomass + \left( v_{1\%} - v_{0\%} \right) \right]$

$unaccounted = 25342$ **$CO_2$ unaccounted for, µl**

$$unaccounted_{\%} := \frac{unaccounted \cdot 100}{v_{co2}}$$

$unaccounted_{\%} = 32.3$ **percent $CO_2$ unaccounted for**

## Appendix H: Regression Analysis

The Statistix™ statistics package outputs were used to perform regression analyses on the effects of each level of jet fuel on the overall levels of nitrates and phosphates in the soils after each experiment's run. The raw data used to perform these analyses follows these analyses.

## NITRATES

UNWEIGHTED LEAST SQUARES LINEAR REGRESSION OF A_NO3 (SOIL A)

| PREDICTOR VARIABLES | COEFFICIENT | STD ERROR | STUDENT'S T | P . |
|---|---|---|---|---|
| CONSTANT | 18.7823 | 1.48617 | 12.64 | 0.0000 |
| FUEL | -10.5426 | 2.56135 | -4.12 | 0.0021 |

| | | | |
|---|---|---|---|
| R-SQUARED | 0.6288 | RESIDUAL MEAN SQUARE (MSE) | 15.9202 |
| ADJUSTED R-SQUARED | 0.5917 | STANDARD ERROR OF ESTIMATE | 3.99002 |

| SOURCE | DF | SS | MS | F | P . |
|---|---|---|---|---|---|
| REGRESSION | 1 | 269.714 | 269.714 | 16.94 | 0.0021 |
| RESIDUAL | 10 | 159.202 | 15.9202 | | |
| TOTAL | 11 | 428.917 | | | |

UNWEIGHTED LEAST SQUARES LINEAR REGRESSION OF B_NO3 (SOIL B)

| PREDICTOR VARIABLES | COEFFICIENT | STD ERROR | STUDENT'S T | P . |
|---|---|---|---|---|
| CONSTANT | 11.6827 | 1.49626 | 7.81 | 0.0000 |
| FUEL | -5.38462 | 2.57873 | -2.09 | 0.0633 |

| | | | |
|---|---|---|---|
| R-SQUARED | 0.3036 | RESIDUAL MEAN SQUARE (MSE) | 16.1370 |
| ADJUSTED R-SQUARED | 0.2340 | STANDARD ERROR OF ESTIMATE | 4.01709 |

| SOURCE | DF | SS | MS | F | P . |
|---|---|---|---|---|---|
| REGRESSION | 1 | 70.3590 | 70.3590 | 4.36 | 0.0633 |
| RESIDUAL | 10 | 161.370 | 16.1370 | | |
| TOTAL | 11 | 231.729 | | | |

UNWEIGHTED LEAST SQUARES LINEAR REGRESSION OF C_NO3 (SOIL C)

| PREDICTOR VARIABLES | COEFFICIENT | STD ERROR | STUDENT'S T | P . |
|---|---|---|---|---|
| CONSTANT | 21.3235 | 2.13729 | 9.98 | 0.0000 |
| FUEL | -12.2459 | 3.68353 | -3.32 | 0.0077 |

| | | | |
|---|---|---|---|
| R-SQUARED | 0.5250 | RESIDUAL MEAN SQUARE (MSE) | 32.9260 |
| ADJUSTED R-SQUARED | 0.4775 | STANDARD ERROR OF ESTIMATE | 5.73812 |

| SOURCE | DF | SS | MS | F | P . |
|---|---|---|---|---|---|
| REGRESSION | 1 | 363.907 | 363.907 | 11.05 | 0.0077 |
| RESIDUAL | 10 | 329.260 | 32.9260 | | |
| TOTAL | 11 | 693.167 | | | |

## PHOSPHATES

UNWEIGHTED LEAST SQUARES LINEAR REGRESSION OF A_PO4 (SOIL A)

| PREDICTOR VARIABLES | COEFFICIENT | STD ERROR | STUDENT'S T | P . |
|---|---|---|---|---|
| CONSTANT | 55.6821 | 5.85150 | 9.52 | 0.0000 |
| FUEL | -12.1786 | 10.0848 | -1.21 | 0.2550 |

| | | | |
|---|---|---|---|
| R-SQUARED | 0.1273 | RESIDUAL MEAN SQUARE (MSE) | 246.800 |
| ADJUSTED R-SQUARED | 0.0400 | STANDARD ERROR OF ESTIMATE | 15.7099 |

| SOURCE | DF | SS | MS | F | P . |
|---|---|---|---|---|---|
| REGRESSION | 1 | 359.917 | 359.917 | 1.46 | 0.2550 |
| RESIDUAL | 10 | 2468.00 | 246.800 | | |
| TOTAL | 11 | 2827.92 | | | |

UNWEIGHTED LEAST SQUARES LINEAR REGRESSION OF B_PO4 (SOIL B)

| PREDICTOR VARIABLES | COEFFICIENT | STD ERROR | STUDENT'S T | P . |
|---|---|---|---|---|
| CONSTANT | 97.4173 | 10.4605 | 9.31 | 0.0000 |
| FUEL | 6.88462 | 18.0281 | 0.38 | 0.7105 |

| | | | |
|---|---|---|---|
| R-SQUARED | 0.0144 | RESIDUAL MEAN SQUARE (MSE) | 788.699 |
| ADJUSTED R-SQUARED | -0.0842 | STANDARD ERROR OF ESTIMATE | 28.0838 |

| SOURCE | DF | SS | MS | F | P . |
|---|---|---|---|---|---|
| REGRESSION | 1 | 115.019 | 115.019 | 0.15 | 0.7105 |
| RESIDUAL | 10 | 7886.99 | 788.699 | | |
| TOTAL | 11 | 8002.01 | | | |

UNWEIGHTED LEAST SQUARES LINEAR REGRESSION OF C_PO4 (SOIL C)

| PREDICTOR VARIABLES | COEFFICIENT | STD ERROR | STUDENT'S T | P . |
|---|---|---|---|---|
| CONSTANT | 122.079 | 17.3588 | 7.03 | 0.0000 |
| FUEL | -35.9203 | 29.9171 | -1.20 | 0.2576 |

| | | | |
|---|---|---|---|
| R-SQUARED | 0.1260 | RESIDUAL MEAN SQUARE (MSE) | 2171.94 |
| ADJUSTED R-SQUARED | 0.0386 | STANDARD ERROR OF ESTIMATE | 46.6041 |

| SOURCE | DF | SS | MS | F | P . |
|---|---|---|---|---|---|
| REGRESSION | 1 | 3131.06 | 3131.06 | 1.44 | 0.2576 |
| RESIDUAL | 10 | 21719.4 | 2171.94 | | |
| TOTAL | 11 | 24850.5 | | | |